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STUDIES ON THE FUNCTION OF THE INTESTINAL MUSCULATURE

I. Longitudinal Muscle of the Rabbit (Gradients)

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Received for publication August 4, 1928

Historical. It is not necessary for us more than briefly to mention the outstanding milestones in the development of our knowledge of the physiology of the gastro-intestinal musculature, as related to our thesis.

Digestion goes on in the stomach and intestine when the vagi and splanchnics have been severed (Krehl, 1892; Cannon, 1906; Rubaschou, 1912).

Peristalsis continues in excised segments of stomach and intestine when they are perfused with or simply placed in oxygenated or aerated Locke's solution (Magnus and many others).

Nerve tissue is not necessary for the contraction of longitudinal and circular muscle (Bayliss and Starling, 1899; Magnus, 1905; Gunn and Underhill, 1914; Alvarez and Mahoney, 1922).

The gastro-intestinal muscle is autonomous.

There are gradients of rate, amplitude, tone, vascularity, metabolism and catalysis in the gastro-intestinal musculature. These have been demonstrated chiefly by Alvarez and his associates. It is to the study of these phenomena that this investigation has been directed.

Method. The following observations were made by an adaptation of the Magnus method, the greatest care being taken to select animals and to prevent shock to the tissues which Alvarez has shown is occasioned by rough handling. The segments of intestine were all cut the same length, avoiding the possibility of stretching. The lower ends of the segments were held in a glass cell of Locke's solution by metal hooks attached to a glass supporting rod bent at right angles and clamped to a stand. The upper ends were attached by metal hooks to threads passing over pulleys

and counterbalanced by lead shot. Levers attached to the pulleys were arranged so they wrote on the moving drum, one directly above the other. Surrounding the inner cell through which oxygen bubbled at the rate of 20 to 30 per minute was a beaker water bath maintained at the desired temperature by a small Bunsen burner.

The rate gradient in the duodenum, small intestine and colon. Alvarez points out that the rhythmic rate of the contractions of the longitudinal muscle of the intestinal tube varies inversely as the distance from the pylorus. That is, the duodenum beats faster than the jejunum, the jejunum faster than the upper ileum, the upper ileum faster than the middle and lower ileum, and the lower ileum faster than the colon. We have been able to demonstrate this fact by numerous experiments which have been very carefully controlled. For example, in a series of ten animals in which the contractions of only one segment of each animal were recorded a gradient of rate, inversely as the distance from the pylorus, was always found. There were three duodenal, two jejunal, three lower ileal and two ascending colon segments observed. The duodenal and jejunal rates were practically the same.

In a series of eleven animals in which the contractions of two or more segments on each animal were recorded a rate gradient, inversely as the distance from the pylorus, was shown in all but two. In these two, the upper and lower ileal rates were practically the same (10 and 12.5 respectively). There was always a gradient between duodenum and lower ileum. We have been able to follow this gradient of rate in long stretches of tracings; sometimes for periods of three or four hours at a time; sufficient to enable us to state beyond question that, with all conditions of observation as nearly normal as they can be made, it is characteristic of the intestinal rhythm.

It is of interest to note that when all the duodenal, jejunal, and ileal rates taken at random are respectively added together and the average number of contractions determined, a gradient is still seen. Each segment has a rhythmic range within which it almost always falls (table 1).

Amplitude gradient. Alvarez believes the amplitude of contraction of the longitudinal muscle increases directly as the distance from the pylorus; in other words, in inverse ratio to the rate gradient. We have been able to demonstrate this finding in quite a number of records but it is not as constant as the rate gradient; eight times in eleven animals. The duodenum always beats with less amplitude per unit of length than the lower ileum but we very frequently find that the amplitude of the jejunum varies considerably. Sometimes it is smaller than the duodenum, experiment 18, figure 1, while at other times it is greater than the ileum, experiment 16, figure 2. Table 2 shows how the amplitudes of contraction, as recorded on the smoked drum, vary with the length of the segment.

Tone gradient. Alvarez observed that "when a piece of the duodenum or jejunum of the cat is cut out it tends to contract down to perhaps half its original length and the ends roll over so as to form little cuffs. A piece of lower ileum may even lengthen a little and its cuffs if any form at all are narrow as compared with those of the upper bowel." He calls attention to W. Wolf's description in 1902 of variations in the cuffing at the different intestinal levels as well as to the observations of Trendelenburg (1917) that there is a tendency for a loop of the guinea-pig intestine to show greater contraction at the orad than at the caudad end. Trendelenburg suggested that this gradient might account for the direction of peristalsis. We have illustrated this by actual drawings from segments lying in warm Locke's solution. From these drawings it is observed that

TABLE 1

INTESTINAL SEGMENT CONTRACTING	RATE OF CONTRACTIONS PER MINUTE	NUMBER OF ANIMALS OBSERVED
Duodenum	14.5	16
Jejunum	13.0	5
Upper ileum	11.4	8
Lower ileum	10.8	15
Ascending colon	6.0	2

TABLE 2

NUMBER OF RABBITS OBSERVED	INTESTINAL SEGMENT CONTRACTING	AVERAGE AMPLITUDE OF CONTRACTION PER MINUTE	LENGTH OF SEGMENT
			mm.
4	Duodenum	22.3	30
8	Duodenum	21.0	25
3	Duodenum	13.3	15

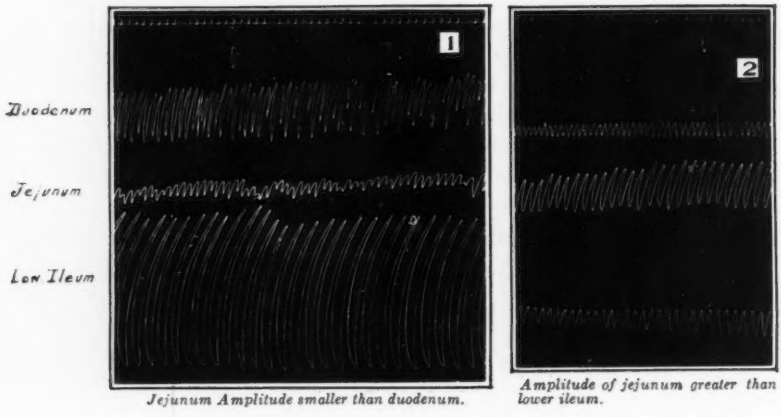
the cuffing is greater at the proximal than at the distal end of the segment. As Alvarez points out, it is easy to tell which end is orad and which aborad by this means.

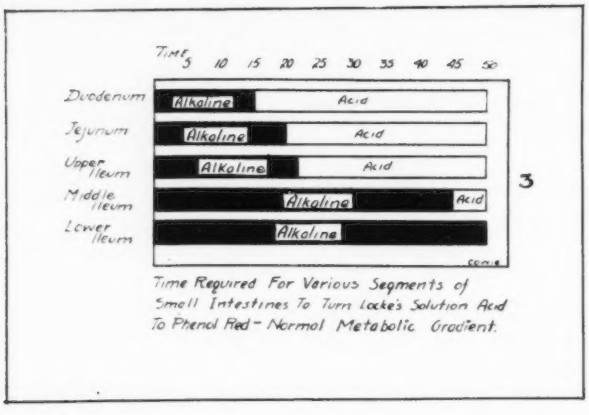
As a further illustration of there being a gradient of tone, we may call attention to our common observation, in experiments that have extended over several hours, that the lower segments of the bowel become flaccid and lose their original shape sooner than the upper segments.

A change in the degree of cuffing is observed when the colon is reached. In segments cut from the ascending colon near its cecal end, we observed as did Alvarez, that the cuffing is more marked than that of the lower ileum indicating a greater tone in the longitudinal muscle at this point. It will be remembered that the longitudinal muscle of the colon does not

completely surround the gut but lies in three bands along the intestinal wall.

The vascular gradient. It has long been known that the blood supply to the upper intestine is greater than it is to the colon. In an article by Monks in 1903 there is a series of six drawings of the vascular supply





Figs. 1 to 3

to the different segments of the small intestine. From these drawings one can see that there is a gradient. Monks states that "the vessels grow smaller and smaller as we pass downward until the lower third of the gut is reached where they remain about the same size as far as the ileocecal valve." In our drawings done by our clinical artist, Miss Perry,

who knew nothing of this fact, this gradient is well shown. The duodenum is rich in vessels and the vascularity diminishes progressively until the colon is reached.

The metabolic gradient. Alvarez and Starkweather (1918) measured the CO_2 output per unit of weight of tissue (guinea pig) and demonstrated a greater CO_2 production in the duodenum than in the jejunum, upper ileum, and lower ileum respectvely (0.30, 0.23, 0.195, and 0.18). We have shown a metabolic gradient by a very simple method, one that is easily adaptable for class demonstration (fig. 3). By placing equal weights of tissue from different intestinal levels in equal amounts of Locke's solution to which phenol red had been added as an indicator, and by supplying a sufficient amount of O_2, we observed that the upper segments produced acid (CO_2) at a faster rate than the lower segments. The duodenum turned acid in 15 minutes, the jejunum in 20 minutes, the upper ileum in 22.5 minutes, the middle ileum in 45 minutes, while the lower ileum preparation was still alkaline in 50 minutes, when the experiment was terminated. We thought this was an original method. Several months later we found that as early as the middle of the last century observers, using litmus as an indicator, found that skeletal muscle turned acid on work. However, no precautions were taken to supply O_2 in their experiments. We did this in our experiments because Fletcher and Hopkins showed that under anaerobic conditions even resting muscle produced lactic acid, but in room air or in oxygen very little, if any, lactic acid was formed. Accordingly lactic acid would not be formed in our experiment and the acid produced could be accredited to CO_2, (H_2CO_3).

CONCLUSIONS

1. Evidence is produced confirming the observations of Alvarez and his co-workers that there is a rate, amplitude, tone, vascular, and metabolic gradient in the normal rabbit's intestine as demonstrated by excised segments from the various intestinal levels contracting in oxygenated Locke's solution and recording on the kymograph.

2. These phenomena are shown to be produced by the contraction of longitudinal muscle.

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STUDIES ON THE FUNCTION OF THE INTESTINAL MUSCULATURE

II. Longitudinal Muscle of the Rabbit. Analysis of Curves Produced by Contraction of Excised Segments in Oxygenated Locke's Solution

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Received for publication August 4, 1928

In our first study (previous paper) we have confirmed the observations of Alvarez and his co-workers that there is a rhythmic amplitude, tone, vascular and metabolic gradient. In this report we endeavor to analyze the curves produced by the contraction of longitudinal muscle in oxygenated Locke's solution.

Primary contractions. In the accompanying tracings it will be seen that the rhythmic contractions are of two types, one in which the amplitudes of contractions are more or less of equal length (expt. 8, fig. 1). The other in which the amplitudes of contraction gradually increase in size to a peak, then decline (expt. 17, fig. 2). In these two types it will be further observed that the rhythmic contractions remain on the same horizontal plane, they never leave the base line. The point of maximum relaxation has not changed. That is, they all relax to the same point regardless of the amplitude. There is still another type of contraction. This is illustrated in experiment 19, figure 3. It is characterized by gradually increasing, followed by gradually decreasing amplitudes of contraction below as well as above the base line, thus presenting a spindle appearance. A line drawn through the center of this contraction complex we regard as the base line.

We have designated these as primary contractions a, b and c. They are rhythmic in nature. It is with these types of contractions that a rhythmic rate gradient has been demonstrated.

Secondary contractions. This group of contractions is shown in experiment 22, figure 4 (jejunum and duodenum). Any of the primary types of rhythmic contractions may be superimposed upon a rhythmic wave of contracture which gradually leaves and returns to the base line. We interpret this form of contracture as being due to a change in the tone of the

[1] Presented at the Washington meeting of the American Pediatric Society, May 1, 1928.

longitudinal muscle. In order for a wave to leave the base line in either direction there must be a change in the muscle tone. If the tone is not sustained, the rhythmic contractions will fall below the base line; if the tone is increased, the rhythmic contractions will rise above the base line. These secondary contractions last from 10 to 30 seconds.

Tertiary contractions. It will be seen in experiment 8, figure 5, that there is a series of secondary tone waves superimposed on a third tone wave. This we have called a tertiary wave of contraction. It is present in practically all normal curves that run for many hours. These waves last from a few minutes to an hour or more. An eight foot tracing, for example, which may require from 1½ to 2½ hours to be completed may show only two of these waves.

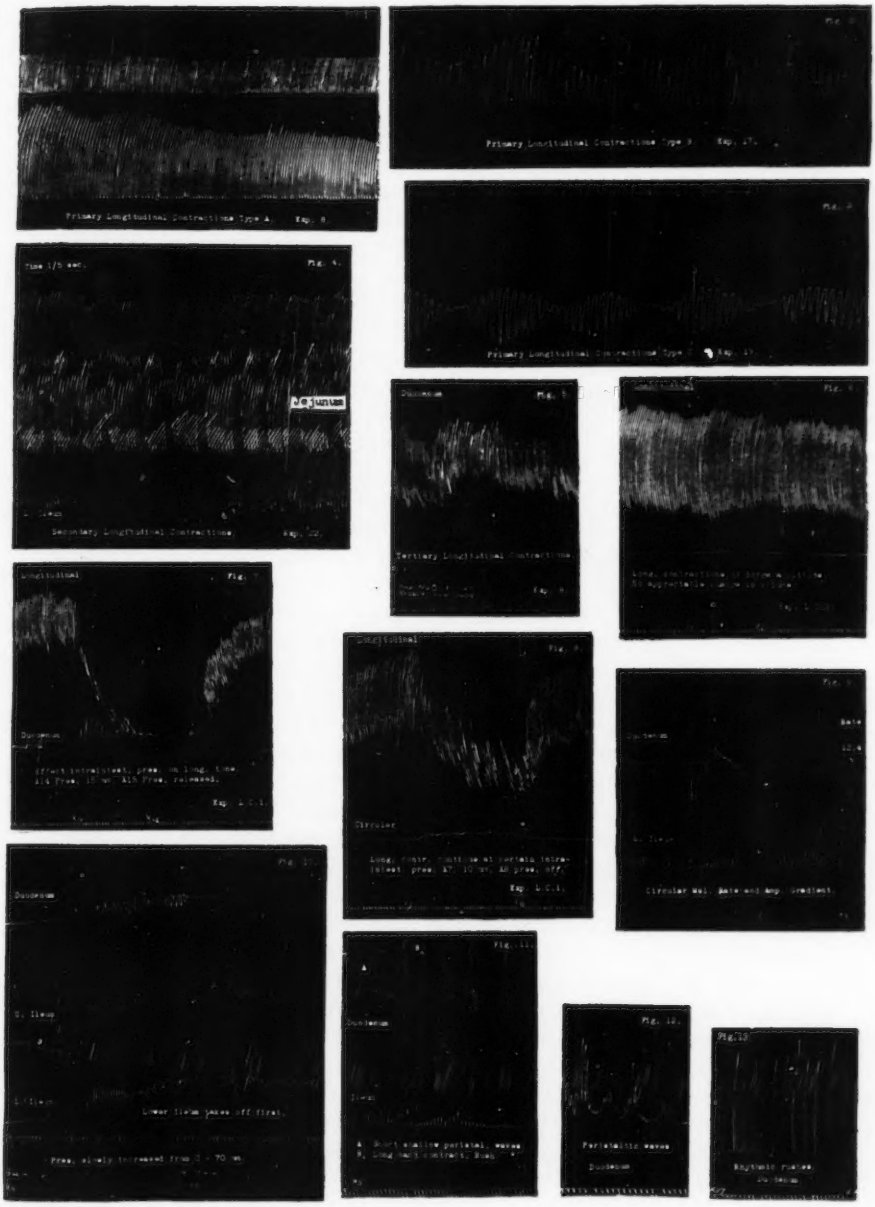
The tertiary wave does not necessarily return to the base line. It may fall to within several millimeters of the base and another wave start upward, or it may fall below the base and again start upward. The most common wave we see of this type rises quite rapidly, is maintained for from 10 minutes to an hour, then gradually falls to rise again less abruptly to a decreased height. Each succeeding rise is lower than the preceding.

Discussion. *Primary types.* In the first primary curve (type a), (fig. 18) we note that the rate is regular and rhythmic and the amplitude is practically the same for each contraction; the systolic, diastolic and mean tones remain the same. This type of contraction may be found in all segments and follows a gradient.

In the second primary curve (type b) (fig. 18) the rate is regular and rhythmic but the amplitude progressively increases to a peak and then declines; the systolic and mean tones gradually increase and then gradually diminish, while the diastolic tone remains unchanged. This type of curve may also be found in all segments.

In the third primary curve (type c) the rate is regular and rhythmic but the amplitude progressively increases and diminishes; the systolic tone gradually increases and diminishes while the diastolic tone gradually diminishes to a low point and then gradually increases; the mean tone remains unchanged. This type of wave we have encountered more frequently in the duodenum but it may be found in the lower segments.

Secondary types. In the secondary contractions, the rhythmic contractions may be any of the three primary types, (fig. 18) but in addition, they are superimposed on a tone wave of contracture in which the systolic, the mean, and the diastolic tones gradually increase and then diminish, experiment 22, figure 4, that is, gradually leave and then return to the base line. In many of our experiments secondary contractions are more frequent and for this reason are of shorter duration in the duodenum than in the lower segments and therefore suggest a gradient. We found this type of curve more frequently at the beginning of an experiment, particularly when the



Figs. 1 to 13

Magnus method was used. Our explanation for this phenomenon will be discussed later (study V).

Tertiary types. Tertiary contractions are secondary tone contractions, all types, superimposed on a third or tertiary tone wave of contracture which may be made up of many secondary contraction waves and may continue for long periods of time. The secondary tone waves rise higher and higher until the peak of the tertiary tone wave is reached where they may be maintained or gradually fall to a lower level. They may, or they may not, return to the base line, or they may fall below it before a succeeding tertiary tone wave is initiated.

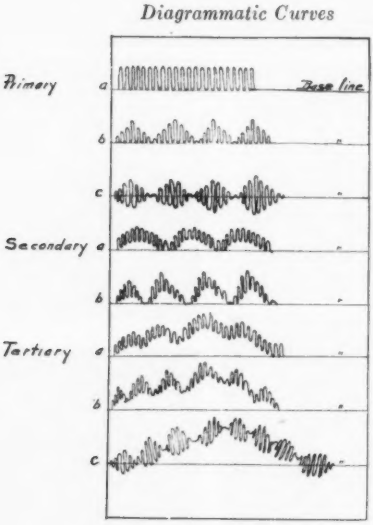


Fig. 18

CONCLUSIONS. 1. An analysis of the various types of curves produced by the contraction of the longitudinal muscle of excised segments of the rabbit's intestine in oxygenated Locke's solution is given.

2. There are three primary types of contractions of longitudinal muscle.

a. Those in which the amplitudes of contraction are of uniform height and always return to the base line.

b. Those in which the amplitudes of contraction form a complex by gradually increasing to a peak then declining but always returning to the base line.

c. Those in which a spindle complex is formed by the amplitudes of contraction gradually increasing and gradually falling below, as well as above the base line, as if two b complexes were placed base to base.

3. There are three secondary types of contractions of longitudinal muscle, produced by any of the primary types of rhythmic contraction being superimposed upon a rhythmic wave of contracture which gradually leaves and returns to the base line.

4. There are tertiary types of contractions of the longitudinal muscle. These are composed of a series of secondary tone waves superimposed upon a tertiary tone wave which may endure for a few minutes or for an hour or more. This tertiary tone wave may or may not return to the base line or it may fall below the base line.

III. The Effect of Longitudinal Muscle Contraction and Relaxation

The effect of longitudinal muscle contraction and relaxation on the lumen of the excised intestinal segment. As the muscle contracts the lumen changes in shape but the capacity and volume remain the same. The segment shortens, the lumen becomes wide. As the muscle relaxes the segment becomes longer and the lumen narrower. This is shown in figure 6, experiment L.C. 3. The longitudinal contractions are of quite large amplitude. Nevertheless, there are no appreciable changes in the volume capacity of the inside of the gut.

The effect of longitudinal muscle contraction and relaxation on the contents of the excised segment. It is not difficult to see by the direct observation of a segment contracting in oxygenated Locke's solution, particularly with a thin segment, that the particles move backward and forward without advancing beyond the segment. That is, a segment closed at one end may contain particles of feces which move about in all directions, especially forward and backward without leaving the segment. The same may be more regularly seen in the intact animal where no manipulation has been operating. The intestine is seen to move up and down over scybalae which may change their position from side to side, or up and down. This lends evidence to the idea that longitudinal muscle contractions help mix the intestinal contents. We think this necessarily operates by bringing the center of the contents to the periphery, or to the wall of the intestine, thus incidentally presenting a greater surface for absorption.

With the animal opened under Locke's solution,[2] one's attention is first called to the larger intestinal movements; the active vermicular movements, where a large portion of the gut may be seen to plunge into the depths of the abdomen and as quickly rise again in a swinging to and fro manner; or, where a large segment may be seen to swing laterally, to one side, then as quickly straighten out again but never swinging to the opposite side, depending we think upon the position of the mesentery.

These are longitudinal muscle phenomena. In segments filled with feces or bubbles of air, these vigorous movements can be easily seen to slide up and down over the intestinal contents oftentimes without changing their position. Even a gas bubble may remain at a fairly fixed point while the longitudinal muscle contracts and relaxes above it, the segment blanching and blushing meanwhile. This action in itself would present a greater absorption surface without the intestinal contents moving. These are the pendulum movements, Pendelbewegung, of other observers and these are the movements which record great amplitudes of contraction on the moving drum. They are greater in the lower ileum and gradually lessen in their

[2] Soon after a blow at the base of the skull.

swinging propensities as the higher segments are reached. In the duodenum, probably because of its more fixed position, pendulum movements may be almost imperceptible.

The effect of longitudinal muscle contraction and relaxation on the blood supply of the segment. In the intact animals, we have observed that during longitudinal contraction the intestine becomes paler while during relaxation the color returns. We interpret this as due to compression and relaxation of the blood vessels. In other words, contraction induces anemia of the segment, relaxation induces euemia—somewhat as a secondary heart would do.

The relationship of longitudinal contraction to longitudinal tone. In the secondary contractions the point of greatest longitudinal relaxation is lifted higher and higher from the base line, and the segment gets shorter, that is, the tone increases as the peak of the curve is reached, and often the amplitudes of the contractions become smaller. Sometimes there seems to be this relationship between longitudinal contraction and tone. At other times we do not see this relationship. For example, one might think that where the rate is fast and the amplitude is small, the tone would be greater; and vice versa, when the rate is slow and the amplitude great, the tone is least. Indeed, this is very often the case and we know from other evidence presented that the duodenal tone is very much greater than the lower ileal tone, and that the amplitude of the lower ileum is usually greatest.

The effect of longitudinal contraction and relaxation on the propulsion of aliment. In a previous paragraph we have called attention to the observation that particles in the lumen of the intestine are not pushed onward by the longitudinal contractions. This is particularly well shown in our studies on circular muscle where it is regularly seen that aliment progresses onward only when advancing circular contractions take place. There is a definite time relationship between circular contraction and the drop in longitudinal tone, as will be shown later. The question arises, which initiates the other; or does either have anything to do with the initiation of contraction of the other. Whatever the answer may be, it seems certain that longitudinal contraction has no direct relation to propulsion of aliment, but may have an indirect relationship due to a possible effect on circular contractions.

The effect of intra-intestinal pressure on the contraction of longitudinal muscle. With the upper end of an intestinal segment closed and attached to the pulley of a recording lever and the lower end slipped over a glass tube admitting water under pressure control,[3] it can be regularly demonstrated that increasing the pressure to a certain point stops the longitudinal contraction; decreasing the pressure particularly taking the presssure away

[3] Trendelenburg method.

restores rhythmic longitudinal contraction and increases *its* tone (expt. L.C.1, fig. 7). There is, however, a certain intra-intestinal pressure at which longitudinal contractions continue but they are altered. For example, in experiment L.C.1, figure 8, it will be noticed that when 10 mm. of water pressure was applied (at 7) the contractions continue but instead of being the primary type, as previously described, they are now of the secondary type. When the pressure is removed they again become the primary type. Incidentally, attention is called to the presence of circular contractions when the secondary longitudinal contractions begin their phase of greatest relaxation. Longitudinal tone is lowered when pressure is applied under the conditions noted above.

Conclusions. 1. An attempt is made to explain the effects of longitudinal contraction and relaxation of excised segments of rabbit's intestine and of corresponding segments in the intact animal immediately after being killed by a blow at the base of the skull.

2. Contraction of longitudinal muscle shortens the segment, and widens the lumen. Relaxation lengthens the segment and narrows the lumen. There are no appreciable changes in the volume capacity produced by these movements.

3. Longitudinal contraction and relaxation in excised segments and in the intact animal are non-propulsive. They seem to serve the purpose of exposing a greater surface for absorption and act as a secondary heart alternately blanching and blushing the intestine. Longitudinal contractions may have an indirect effect on the propulsion of aliment because of their having a possible effect on circular contractions.

4. Intra-intestinal pressure or tension, depending on its degree, stops longitudinal muscle contraction. There is, however, a certain pressure at which longitudinal contractions continue but they are altered in character. A primary type of contraction may thus be changed to a secondary type. When the pressure is removed, the original type of primary contraction is usually resumed.

IV. Circular Muscle of the Rabbit

Our previous communications have been concerned with the phenomena of the contractions of longitudinal muscle of the various intestinal segments of the rabbit. The present report deals with certain phenomena encountered during the study of contractions of the circular muscle.

Method. The apparatus we have finally adopted after many trials of several others is chiefly after the original Trendelenberg method (fig. 19). The segments were immersed in oxygenated Locke's solution at a constant temperature of 37°C. Longitudinal contractions were recorded by a pulley lever over which a thread passed attached to the closed orad end of the intestinal segment. Circular contractions were recorded by air

displacement through a glass tube to a tambour; thus differing from the Trendelenberg apparatus. Tension on the inside of the gut could be increased or decreased at will by increasing or decreasing the hydrostatic pressure.

With this apparatus, when no positive pressure is operating longitudinal muscle contractions are recorded exactly as they are with the Magnus method used in our previous reports. No differences are seen in parallel experiments with both methods. When positive hydrostatic pressure is operating, circular contractions are recorded in addition to the longitudinal

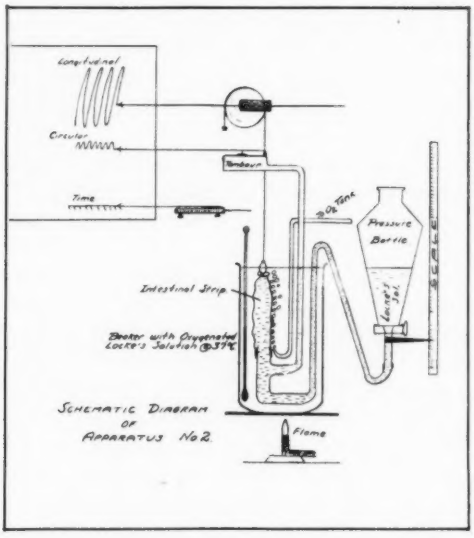


Fig. 19

contractions and the simultaneous relationships between these separate contractions may be easily followed.

Our first interest was to determine whether there is a gradient with circular muscle similar to that obtained with longitudinal muscle. The results of our observations are as follows:

Rate gradient of circular muscle. We have been able to demonstrate a rate gradient in practically all of our experiments with normal circular muscle. Figure 9 illustrates this very well. Table 1 records the number of contractions per minute in one of these (expt. 21, A and B). This experiment ran for two hours.

Amplitude gradient. With our present method which is a displacement system we are unable to regularly demonstrate an amplitude gradient. It

is occasionally seen as shown in figure 9 but it is just as frequently the reverse.

Tone gradient of circular muscle. In a previous report we have called attention to the work of Crane and Henderson in which they found that the stretching stimulus awakened a peristaltic response at a lower pressure in the ileum than in the higher segments. "There is less difference between ileum and jejunum than between ileum and duodenum." Trendelenberg

TABLE 1

Comparative rate of circular contraction of upper duodenum and lower ileum

EXPERIMENT NUMBER	HYDROSTATIC PRESSURE		RATE OF CONTRACTION PER MINUTE		PERIOD OF OBSERVATION	
	Duodenum	Lower ileum	Duodenum	Lower ileum	Minutes	Seconds
	mm.	*mm.*				
21 A	0*	0*	16.8	12.9	3	20
	5	5	19.0	22.0	2	35
	10	10	15.0	13.9	4	35
	15	15	16.0	12.8	2	35
	20	20	18.9	15.4	2	35
	10	10	14.3	12.3	3	
	15	15	16.8	15.4	10	45
21 B	0*	0*	12.4	10.0	7	
	5	5	12.0	10.3	2	25
	10	10	12.8	11.7	3	30
	15	15	14.1	11.8	6	25
	20	20	16.3	11.6	3	
	25	25	17.0	14.1	1	35
	30	30	29.1	18.8	0	35
	40	40	15.0	15.0	0	40
	20	20	16.9	13.6	1	50
	40	40	19.5	17.3	2	15

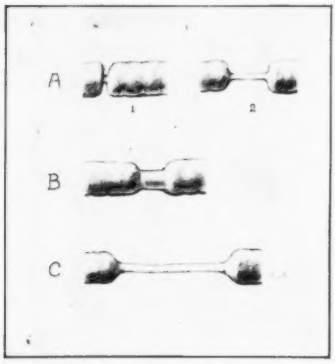
* There is really some pressure here due to slight dilatation of the segment. The zero point is just below the first signs of filling of the segment. In this experiment, the zero mark showed slight filling of the segment. In segments with slight negative pressure recorded, no contractions are seen. Segments lying empty in a dish of Locke's solution show no circular contractions.

pointed out (1917) that the circular muscle at the pyloric end of a segment has a higher tone than at the cecal end. Alvarez also demonstrates this and Luciani observes "That the rate and forces of the intestinal movements diminish regularly from jejunum to ileum."

In experiments where the intra-intestinal pressure is very slowly increased it is observed that the contractions of the lower ileum begin first (fig. 10). At times it may be several minutes before the duodenum begins to contract on a gradually increasing pressure. In our first experiments of

which 21 A and B, table 1, is an illustration, we were not aware of the importance of slowly increasing the pressure as was done by Trendelenberg (1 mm. in 2 to 4 seconds) and later emphasized by Crane and Henderson. We increased the pressure by elevating the pressure bottle five millimeters at a time, paying no attention to whether this was accomplished slowly or rapidly.

Crane and Henderson employed a clock mechanism for raising the pressure bottle and adopted the plan of increasing the pressure 1 mm. in 2 or 3 seconds. We obtained the same result by raising the bottle with a fishing line passing over a pulley and attached to a micrometer screw carefully turned by hand, or by the use of a rack and pinion as was employed by Trendelenberg. By taking this precaution, we find that the different segments take off in the following sequence: lower ileum, upper ileum, duodenum. This is well illustrated in figure 10. The same thing does happen when the pressure is raised quickly (table 2); but the successive take-offs are not so uniformly demonstrated. When the pressure is raised quickly, the take-offs occur in more rapid sequence and if the drum is moving very slowly, this character may not be recorded. These phenomena occur quite regularly in the respective types of experiments. Our observations, accordingly, support the opinion that the duodenum is less sensitive to pressure than the lower segments.



A Non Traveling Circular Muscle Contractions
B Traveling Peristaltic Contractions
C Traveling Peristaltic Rush Waves.

Fig. 20

Types of contraction of circular muscle. When an intestinal segment is placed under tension in warm oxygenated Locke's solution the following types of contraction may be seen.

1. *A small deep non-traveling circular band of contraction* is occasionally seen (fig. 20, A). The width of this contraction may not be more than 2 or 3 mm. (1), or it may be a centimeter or more long (2). These contractions apparently obliterate the lumen of the segment. They are almost always seen at the orad end of the segment; rarely at the caudad end. These contractions are doubtless analogous to the rhythmic segmentations described by Cannon in the cat, white rat, and dog. They, however, are not rhythmic. We have occasionally observed a series of two to four of

these segmenting, non-traveling, non-propulsive contractions, occurring one after the other in not more than a minute's time while watching a portion of the ileum of the intact animal under Locke's solution. They continue in this position for a minute or two then relax and the intestine assumes its precontraction state. Cannon says "The (rhythmic) segmenting movements I have never seen in the rabbit." These contractions may occur at long or short intervals in the excised segments or in the intact animal opened under Locke's solution.[4] In the segment or in the intact animal rarely if ever can the intestinal contents be seen to be propelled onward to any marked degree by this type of contraction.

The wide band stationary contraction, A2, not infrequently shows at either end of the contraction, an invagination that makes it appear like a narrow circular contraction. This condition may remain for several minutes during which time the invaginated ends may be pulled apart with the slightest tension and reveal the contracted band in its original position. There is, however, no definite rhythm to these contractions. One may watch a given segment for quite a long time without seeing one in the same neighborhood, but may observe one or two taking place in distant segments, while at other times, several may occur in quite rapid succession.

2. *A short shallow traveling band of contraction, figure 20 B.* This contraction may be a very narrow band or a band one centimeter in length. It is almost always wider than type A. It will also be seen to originate at the orad end of the excised segment and travel downwards for 4 or 5 cm. As the band of contraction progresses down the segment it does not alter its size to any extent. It maintains fairly well the width it assumed when it began to travel. We designate these as true peristaltic waves. In this type of contraction, the contents are advanced through the lower end of the segment into the cannula and in the intact animal they can be plainly seen to advance to a lower segment.

3. *A long traveling band of contraction, figure 20 C.* In this type of contraction a wave begins in the upper end of the segment and continues to contract without relaxation above. That is, there is a series of progressive contractions without relaxation above. The wave may travel the entire length of the segment so that when it has spent itself the entire segment appears to be in a state of tonic contraction. The segment may have elongated to twice its original length. The lumen has become narrowed almost to obliteration. This continuous contraction progresses from its starting point in the intact animal to a distance of 5 to 10 cm. We designate these as the incomplete rush waves described by Meltzer and Auer in 1907 (p. 259) and as Rollbewegungen by Houckgeest in 1872. Meltzer and Auer describe the peristaltic rush that travels from the duodenum to the cecum

[4] Shortly after a blow at the base of the head.

as a complete rush wave. In this type of contraction the contents are advanced through the lower end of the segment farther into the cannula and with greater force than in type B contractions.

In the intact animal we have seen peristaltic waves start near any of the stationary forms of contractions.

Curves produced by the three types of circular contractions. In our first apparatus we were unable to record the stationary segmenting contractions. With our present apparatus any contraction that appreciably alters the lumen of the segment and by so doing displaces fluid, is recorded on the kymograph. The narrow and wide band stationary contractions produce curves varying from an occasional mere ripple to curves 3 to 5 mm. high or higher. As they are not rhythmic, occurring at wide intervals, and are not recognized in all experiments, they are difficult to differentiate from the dominant traveling rhythmic waves that always respond to an increase in intra-intestinal pressure. They last from 5 to 10 seconds. As the lumen of the segment is always closed at the orad end in this apparatus, fluid can only be displaced in one direction. We can only express an opinion as to the propulsive character of these contractions. In the intact animal, it appears to us that these stationary contractions displace intestinal contents only slightly in either direction. There is apparently no tonus behind to sustain an onward displacement such as we see in the true peristaltic movements. They are more of a haustral nature. Because of the invagination sometimes seen, they make one think of the intussusceptions sometimes met with in autopsies on infants, excepting that they are of less marked degree.

We have called attention to the fact that all of these records are obtained from animals recently killed by a blow at the base of the head. This factor must be carefully considered before drawing too definite conclusions. It may be that happenings depicted by these methods of investigation, while of great interest, do not give the answer as to how the intestines behave during life and in the unopened abdomen.

The short shallow traveling peristaltic bands of contraction produce curves that are almost always distinctly higher than those of the stationary contractions (fig. 11 A). They last several seconds. They not infrequently fail to return to the base line (fig. 12), being followed by one or two succeeding contractions which reach a peak, then decline; not unlike the secondary curves produced by longitudinal muscle. These curves or waves are of shorter duration than the stationary waves and are usually rhythmic.

The long traveling band of contraction or peristaltic rush. These curves (fig. 13) are very characteristic. They usually rise abruptly from the base line to a peak which is frequently rounded, and fall in the same manner. At other times, the curves are notched as in figure 11 B. They last from 5 to 10 seconds. We think they are definitely differentiated from the short

peristaltic waves by their height and their prolonged time period. They vary from 20 to 60 mm. in height. Alvarez illustrates these well.

With the method described we are only able to record circular contrac-

TABLE 2

Showing circular muscle tone variations at different intestinal levels by observing the pressure necessary to initiate contractions, and the time interval

	OBSERVATION NUMBER	HYDROSTATIC PRESSURE		TAKE OFF: Duodenum	TAKE OFF: Upper ileum	TAKE OFF: Lower ileum	REMARKS
		Gradually increased from mm.					
*	28 B x 2	0– 8	Pressure	8.0	5.0	0	
			Seconds	200.0	144.0	1.0	Shows gradient
	25 A x 1	0–20	Pressure	20.0		5.0	
			Seconds	1635.0		90.0	Shows gradient
	28 B x 5	0–30	Pressure	38.0	11.0	9.6	
			Seconds	115.0	34.0	29.0	Shows gradient
	33 B	0–65	Pressure	20.5	16.5	1.0	
			Seconds	61.5	49.5	0	Shows gradient
	28 B x 4	0–70	Pressure	31.0	7.0	1.3	
			Seconds	115.0	34.0	29.0	Shows gradient
		Suddenly increased from mm.					
†	21 A x 1	0– 5	Seconds	60.0		52.5	Shows gradient
	22 x 6	0–10	Seconds	194.0		1.0	Shows gradient
	21 x 8	0–20	Seconds	4.0		3.0	Shows gradient
	34 B	0–30	Seconds	4.0	3.0	0	Shows gradient
	22 x 4	0–40	Seconds	137.0		132.0	Shows gradient
	33 A	0–50	Seconds	2.5	1.5	0.5	Shows gradient
	28 A x 4	0–60	Seconds	10.0	4.0	2.5	Shows gradient
	B x 6	0–70	Seconds	9.0	2.0	5.0	Shows gradient

* In 14 observations, a complete gradient was shown 9 times. A gradient between duodenum and lower ileum, 12 times.

† In 28 observations, a complete gradient was shown 9 times. A gradient between duodenum and lower ileum, 15 times.

tions of segments removed from the intestines. On the other hand, the ocular appearance of these contractions is exactly the same as in the intact animal.

CONCLUSIONS. 1. A method for the simultaneous recording of circular and longitudinal muscle is described.

2. A rate gradient of circular muscle is demonstrated.

3. An amplitude gradient of circular muscle is not constant but is sometimes demonstrated.

4. A tone gradient of circular muscle is demonstrated.

5. The duodenum is less sensitive to pressure than the lower segments.

6. There are three types of circular muscle contraction.

Non-traveling non-propulsive bands of contraction. One narrow and deep; one broad and deep almost obliterating the lumen. We interpret these as analogous to the segmenting contractions described by Cannon in the cat but they are not rhythmic.

A short traveling propulsive band of contraction originating at the orad end of the segment which travels 4 to 5 cm. We interpret these as peristaltic waves.

A long deep traveling propulsive contraction, originating at the orad end of the segment, which continues to contract without relaxation above and may travel the entire length of the segment. We interpret these as the peristaltic rush waves of Meltzer and Auer, the Rollbewegungen of Houckgeest.

7. Characteristic kymographic curves for each type of contraction are illustrated.

V. THE RELATIONSHIP BETWEEN LONGITUDINAL AND CIRCULAR MUSCLE CONTRACTIONS OF THE RABBIT

During our experiments on longitudinal muscle incidental observations on the segments as a whole presented several interesting phenomena which could not be explained at that time.

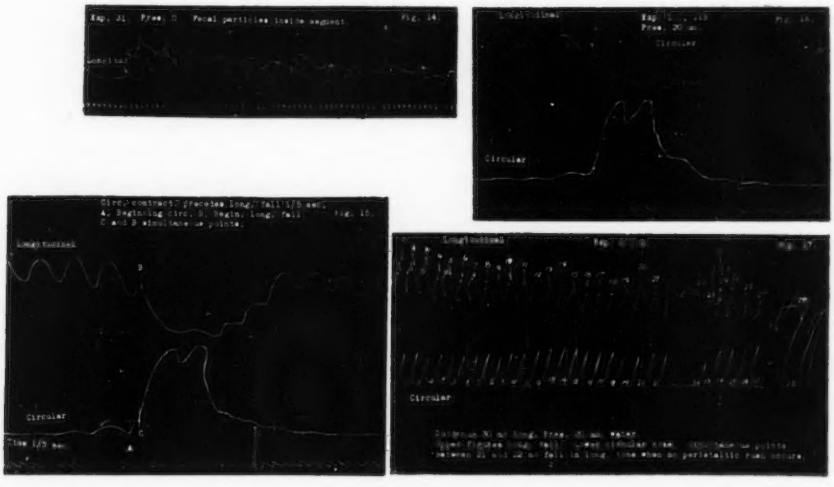
1. In recording longitudinal contractions it was noticed that at the very beginning of the experiment there was a period varying from a few minutes to half an hour or more during which there were tone changes in the segments so extensive that it was at times difficult to keep the recording levers on the smoked drum. During these periods the secondary and tertiary forms of rhythmic contractions were particularly present. Also during these periods circular contractions and true peristaltic waves were observed in the segments themselves but we were not recording them on the drum.

2. After a time these particular tone changes would cease and be replaced by type A primary type contraction, that is, contractions that returned to the base line. This type of contractions would now continue to the end of the experiment, may be two to three hours. During this latter period we noted on constant watching, that no circular contractions or peristaltic movements were visible in the segments (Magnus method).

Later in our observations on circular and longitudinal muscle recording simultaneously we observed that when there was no positive pressure with-

in the segments, frequently the same types of longitudinal contractions were obtained as in the beginning of an experiment with the Magnus method (fig. 14). That is, the same marked tone changes were present, continued for a certain time, and then gave place to type A form of contraction. During this refractory period of the experiment waves of circular contraction were also recorded. They, however, ceased when type A primary contractions began.

Quite early in our studies on longitudinal muscle we observed that these happenings were characteristic of segments which contained particles of fecal matter and that as soon as the lumen of the segments was freed from these



Figs. 14 to 17

fecal contents the primary type of longitudinal rhythm was established. Accordingly if we wished to prevent these marked tone changes in the beginning of an experiment it was only necessary to wash out the segment with Locke's solution and the primary longitudinal rhythm would begin, and vice versa if we wished to induce these marked tone changes it was only necessary to put some pressure or irritation inside the gut. Accordingly in our former experiments we had been dealing with a longitudinal complex which was intimately related to intra-intestinal pressure, tension, or irritation and to circular contractions.

Thus another question presented itself for solution. What relationship has intra-intestinal pressure or tension to circular contractions, peristalsis, longitudinal tone, and to longitudinal rhythmic contractions?

The effect of intra-intestinal pressure or tension on circular muscle contraction. Empty segments lying in a dish of Locke's solution show no circular contractions. Segments filled with feces, lying in a dish of Locke's solution show circular contractions and peristaltic waves so long as the contents are retained. Empty segments attached to our displacement apparatus, show no excursion of the circular muscle recording lever when slight negative pressure is operating. As soon as the pressure is brought above the zero point circular contractions are initiated in the lower segments as are recorded in study IV.

The effect of intra-intestinal pressure or tension on longitudinal tone and rhythm. In simultaneous tracings of longitudinal and circular muscle contractions it is very easy to demonstrate the phenomenon seen in figures 7 and 8. By referring to these it will be observed that when pressure is applied to the inside of the gut and circular contractions are initiated, longitudinal contractions and tone diminish or the longitudinal contractions may cease and the tone fall below the original base line. It will be further observed that when the intra-intestinal pressure is removed and the circular contractions cease to record, longitudinal tone is increasing and gradually reaches the base line where it will remain so long as the intra-intestinal pressure is not operating.

By a careful analysis of many experiments on 20 different animals we have come to the conclusion that circular contraction always precedes the fall in longitudinal tone. This is well shown in figures 14, 15, 16, 17, where it will be seen that the rise in circular tone, or the beginning of circular contraction, *precedes* the fall in longitudinal tone by about one-fifth of a second. We think that the great fall in longitudinal tone is *not necessarily* due to peristaltic rush waves as is the case in experiment L.C. 9, figure 17, between contractions 21 and 22. These illustrate two secondary contractions dependent on intra-intestinal pressure.

This phenomenon is also easy of demonstration by direct observation of the intact animal under Locke's solution. While the plunging up and down movements of the lower ileum are taking place no segmenting, peristaltic or rush waves are seen. This is also the case in the less spectacular longitudinal movements of the higher segments. As soon as a circular contraction begins, the swinging or vermicular motion in that portion of the gut ceases and does not return until the peristaltic waves have spent themselves or the rush wave has traveled far down the gut.

We have previously called attention to the point that there is a certain intra-intestinal pressure during which both circular and longitudinal muscle will be contracting. At this pressure the longitudinal contractions diminish (figs. 8, 14, 17) and change from the primary to the secondary type. When both muscles are contracting longitudinal contractions are always altered. When the secondary longitudinal contractions begin their phase

of greatest relaxation, circular contractions, peristaltic waves and rush waves have already started (fig. 17). Between circular contractions 21 and 22 of this tracing it will be seen that for a minute's time while there were no circular contractions the longitudinal fall in tone is, so to speak, still above the threshold and a second secondary longitudinal complex begins. When the phase of greatest relaxation begins the peristaltic wave 22 has already started. The exact relationship is shown in figures 15 and 16. When both muscles are contracting with slight pressure on or when intra-intestinal irritation is operating, longitudinal contractions are always altered. This is well shown in figure 14, where longitudinal secondary tone waves are terminated by peristaltic rush waves. The entire longitudinal tracing is a series of secondary complexes made up of five or six primary longitudinal contractions superimposed on a tone wave that leaves the base line at the end of the peristaltic wave and returns as the circular wave begins.

The correlation of our findings with those of others will be taken up in a succeeding study.

CONCLUSIONS. 1. The peculiar bizarre happenings in the tracings of longitudinal contractions frequently encountered at the beginning of an experiment seem to meet their explanation in the interpretation of circular muscle contraction.

2. Intra-intestinal pressure or irritation is necessary for the initiation of circular muscle contraction.

3. In simultaneous tracings of longitudinal and circular muscle contractions it is seen that when pressure is applied inside the gut and circular contraction is thus initiated longitudinal contractions and longitudinal tone diminish, or longitudinal contractions may cease and the tone fall below the base line.

4. Circular muscle contraction always precedes the fall in longitudinal muscle tone.

5. The phenomena depicted on the smoked drum are easily demonstrated in the intact animal under Locke's solution soon after being killed by a blow at the base of the skull.

We wish to acknowledge the valuable technical assistance of Mr. Maurice Wurtenberger in the final checking of our observations.

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THERMAL EXCHANGES BETWEEN THE HUMAN BODY AND ITS ATMOSPHERIC ENVIRONMENT

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Thermal processes including heat production within and dissipation from the body have as one of their main functions the maintenance of temperature equilibrium under fluctuating environmental conditions. It is therefore natural to suspect that metabolic processes within and thermal exchanges between the body and its atmospheric environment should be a function of the thermal characteristics of that environment.

From a theoretical consideration, based solely upon purely physical and chemical laws, heat production within and dissipation from the human body should be a function of the thermal condition of the atmospheric environment. In this connection it is interesting to consider the human body as an engine or energy transforming machine. It takes in food or fuel energy in the form of carbohydrates, fats, and proteins, and produces heat and other forms of energy by combination with oxygen. This energy is dissipated from the body as mechanical energy through work performed and, to a greater extent, as heat energy through the avenues of radiation, convection, and evaporation of moisture.

Heat loss by either radiation, convection, or evaporation of moisture must necessarily be in accordance with well-known physical laws. Radiation and convection depend upon the temperature difference between the body and its environment, and also upon the insulation or clothing worn. Evaporation depends upon moisture on the body available for evaporation, the temperature and vapor pressure of the atmosphere, and the clothing worn.

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Ventilating Engineers is interested in metabolism and thermal exchanges as affected by atmospheric conditions in order that the engineer can better supply ventilation conducive to the health and comfort of persons in schools, hospitals, theaters, and other places of assemblage. In cooling an auditorium, equipment must be designed to take care of the heat and moisture produced by the audience.

PREVIOUS WORK. Numerous investigators have given results of research and opinions concerning variation in the metabolic rate with atmospheric conditions. Evidence of this relationship is given by McConnell, Yaglou, and Fulton (1925) who found that the basal metabolism was a minimum in the effective temperature range, 23.9 to 28.3°C. A definite increase in heat production for temperatures above and below this range was shown.

Krogh (1916) shows an invariable increase in metabolism with air temperature for cold-blooded animals. He also shows that for warm-blooded animals there is an increase at both low and high temperatures. Quoting from Rubner, Krogh states that it has been shown often on men and animals that a slight increase in body temperature produces an increase in basal metabolism.

Lusk (1921a) presents data on Rubner showing increase in the metabolism of a guinea pig and a dog subjected to low atmospheric temperatures. There is also an indication of an increase at temperatures of 35 to 40°C.

Lowey (1890), Johansson (1896), Rubner and Lewaschew (1897) state that a low environmental temperature does not produce any increase in the metabolic process unless voluntary or involuntary muscular movements are introduced.

Voit (1878) showed an increase in metabolism with drop in temperature from 15 to 4.4°C. for a man sitting quietly. However, when the temperature fell as low as 4.4°C., shivering was unavoidable. He believed the increase to be a reflex stimulus of cold on the skin which raised the power of the muscle cells to metabolize. Rubner (1902) made confirmatory experiments on a man who did not shiver. An increase in metabolism was apparent at 15°C. over that observed at 23°C.

Wolpert (1898) demonstrates a rise in heat production in man with decreasing atmospheric temperature. He also showed that wind velocity causes an additional increase in heat production.

Lefevre (1922) insists that minimal metabolism is reached when the subject is immersed in a "neutral bath" at a temperature of 35 to 36°C. Benedict and Benedict (1924) found no increase in the metabolism of a woman when first lightly clothed in a room at 16°C. and then placed in a 36°C. bath, but for three men under like conditions there was a distinct rise. Delcourt-Bernard and Mayer (1925) substantiated this rise.

A cold bath at 17°C. is shown by Schapals (1912) to increase the oxygen

consumption 116 per cent above the normal, and a hot bath at 42°C. gives a 15 per cent increase. Lusk (1910) shows an increased heat production of 180 per cent for a person in an 8°C. bath during a period of shivering. Rubner's data from Lusk (1921b) shows an increase in oxygen consumption of 17 per cent in a 44°C. bath. Benedict, Benedict, and Du Bois (1925) exposed 3 men and 2 women first in a room at normal temperature and then in an oilcloth bag through which was driven a blast of very hot dry air. The metabolism increased 5 to 10 per cent in the latter condition.

Hill (1919a) found that metabolism was much higher for a person sitting out of doors especially in cold, windy weather. He also states that Lefevre found that heat production at 5°C. was twice as great as it was at 20°C. with a wind velocity of 1 meter per second (197 ft. per minute).

Hill, Campbell, and Gauvain (1922) showed that the metabolism of children undergoing open air treatment was 20 to 30 per cent higher in winter than in summer. Halfkesbring and Collett (1924) found the basal metabolism of two normal women to be about 5 per cent higher in winter than in summer. Sayers and Davenport (1927) mention in their review of literature on physiological effects of abnormal temperatures and humidities the fact that metabolism increases with high temperatures, and that an increased metabolism of people living in cold climates, as compared to warm, is frequently referred to in the literature.

According to Hill and Campbell (1921) the evidence of increase in metabolism in cold climates seems to be empirical and is really based upon increase in appetite on reaching the cold region. Moss (1923) found an increase in food consumption for persons in high temperatures.

Benedict and Finn (1928) state that it is generally recognized that ingestion of food and muscular activity are the chief factors in the increase of the minimal or basal metabolism of a normal individual.

PRESENT INVESTIGATION. In the investigation here reported the object was to determine the rate of heat production in the body, the rate of heat dissipation to the air, and the differentiation of this loss between that taking place by radiation and convection combined, or sensible heat loss, and that lost by evaporation of moisture or latent heat loss. It was also the purpose of the investigation to determine the rate of moisture loss by evaporation from the skin and lungs.

Subjects. The subjects were students from the University of Pittsburgh and were chosen as representing men of average variation in size and physical condition. Seven subjects participated in the tests, as indicated in table 1. They were all dressed alike wearing light-weight socks, low-cut shoes, B.V.D. underwear, shirt with collar attached, and business suit. The suits were alike, and consisted of typical wool cotton mixed, coat, vest, and trousers of medium weight, provided by and kept at the laboratory for the purpose of the study and for future checking, if desired.

A record was kept of previous activity of the subjects which might affect their reactions during the test. As the object of the investigation was to determine normal thermal reactions of human beings, their regular mode of living was not materially interfered with. It was required that their activity on the previous evening should be normal. They ate a normal breakfast before engaging in the usual college routine. Lunch was eaten between 12 and 1 p.m. after which they reported for tests at about 2 p.m., frequently walking ½ to 1 mile.

During the preliminary 30 minutes and the test period their activity was approximately that of a person normally at rest. They were not permitted to engage in any extreme physical exertion and were required to sit at a table, except while being weighed. No attempt was made to control their posture while seated or the free movement of their limbs. Reading, writing, conversing, observing their body temperature (rectal)

TABLE 1

Physical characteristics of men acting as subjects

SUBJECT	AGE	HEIGHT	WEIGHT	BODY AREA*
	years	*cm.*	*kgm.*	*sq. m.*
H. E. C.	22	181.5	71.8	1.93
V. W. P.	24	177.8	75.0	1.93
K. W. M.	20	175.8	72.3	1.88
A. D. A.	19	168.5	59.9	1.68
F. S. McM.	19	170.1	56.5	1.65
O. D. R.	21	171.4	55.8	1.65
H. E. P.	21	160.0	60.4	1.63

* Values obtained from height-weight chart by Du Bois (1916).

and pulse rate every half-hour, and walking a few steps four times during a test to get on and off the balance constituted the degree of activity. It should be understood that it was not desired to make these tests on men seated absolutely quiet; rather it was endeavored to allow them a reasonable degree of freedom.

Technique. In this investigation two hundred sixty-seven tests were made on human subjects in the psychrometric chambers of the Laboratory of the American Society of Heating and Ventilating Engineers at the Pittsburgh Experiment Station of the U. S. Bureau of Mines. In either of these two rooms described by Houghten and Yagloglou (1923) the desired condition of temperature, humidity, and air motion may be controlled and accurately maintained.

The subjects, on reporting for tests, spent the first 30 minutes in the test room kept at the desired atmospheric condition. During this period,

their pulse rate, body temperature, and weight were recorded. After this preliminary period, the test began and continued for four hours, excepting in a few tests at above 32.2°C. and below 12.8°C. effective temperature, when a shorter period was necessary, due to the inability of the body to maintain thermal equilibrium. Ten minutes after the beginning, and three times thereafter during the test, the subjects were weighed in order to determine the rate of loss in weight. Any changes in weight other than that due to the reactions studied were corrected for. All weighings were made with the subjects wearing their clothing; thus the loss indicated is not affected by unevaporated moisture retained in the clothing and on the body. The expired air of the subjects was measured, sampled, and analyzed in order to determine the rate at which oxygen was consumed and carbon dioxide was produced.

The loss of weight indicated was due to evaporation of moisture from the skin and respiratory tract, and to the difference between the carbon dioxide exhaled and the oxygen consumed. The rate of change in weight due to the difference between the carbon dioxide eliminated and the oxygen consumed in the body was calculated from the quantity and analysis of expired air. The difference between the gaseous exchange and the total loss in weight gives the moisture loss from the skin and lungs. This calculation assumes that carbon dioxide loss through the skin is insignificant.

The rate of heat production in the body was calculated from the carbon dioxide produced and oxygen consumed. Calorific values per liter of oxygen were taken from the table of non-protein respiratory quotients by Zuntz and Schumburg (1901), modified by Lusk (1924). These actual values were reduced by 1 per cent on the assumption that the subjects derived 10 to 20 per cent of their energy from protein. This is in accordance with the standard practice recommended by Du Bois (1927a).

The heat produced in the body is equal to the heat loss so long as the body temperature remains constant. Any rise in body temperature is accompanied by storage of heat in the body equal to its specific heat multiplied by the weight and average temperature rise. Average change in body temperature was taken as the change in rectal temperature, which is only approximately true. However, as the total change was never large, particularly at moderate temperatures, any error resulting is probably insignificant. The total heat loss from the body was taken as the difference between that produced and that stored due to rise in body temperature. The heat loss charged to evaporation was calculated from the weight of moisture evaporated, and the latent heat of evaporation at body temperature. The difference between the total heat loss and the heat loss by evaporation was taken as the heat lost by radiation and convection.

In order to arrive at the true loss in weight to a sufficient degree of accuracy, a bullion balance similar to those employed by the U. S. Treasury Department was used. With this balance it was possible to weigh a dead weight of 91 kgm. to an accuracy of 0.02 gram.

The subjects were weighed while lying down on a tray on one of the balance pans. Difficulty was experienced in weighing a person to the extreme accuracy required because of disturbance due to breathing, heart beat, also because his weight was at no time constant. Because of these disturbances it was only possible to weigh a man to 0.2 gram except by taking time-consuming pains and averaging many weighings. A person loses weight in the temperature range studied at a rate of 0.2 to 3 grams per minute.

When weighing a mass equal to that of an average man, a weight of about 5 grams changes the center of oscillation of the pointer of the balance from one side of the calibrated scale to the other. This fact, together with the slow period of swing of the balance, made it impossible to weigh a man by the usual method of precision weighing, or by adjusting the weights so that successive oscillations of the pointer center over, or very near to, the center of rest or zero of the balance. It was found possible, however, in weighing a man to adjust the weights to less than his weight so that the pointer would begin oscillating over the right hand side of the scale. As he lost weight, successive oscillations would center over points on the scale nearer to the center and then to the left of the center of rest. By timing these swings and determining the center of oscillations, it was possible to arrive at the time when the center of oscillation was over the center of rest or the time when the weights on the balance equaled the subject's weight to plus or minus 5 seconds.

In weighing a subject, the greater part of his mass was balanced with large weights ranging up to 50 pounds, the remainder or less than 2000 grams was balanced by precision metric weights. In successive weighings of a given subject the same large weights were always used and only units of the precision weights were removed to compensate for his loss in weight.

The apparatus, figure 1, used in measuring, sampling, and analyzing the expired air was in a room adjoining the psychrometric chamber. A six-foot flexible rubber hose with a $\frac{3}{4}$-inch bore connected the mouthpiece of the breathing apparatus with the spirometer. An improved valve (Fulton, 1924) developed for use in metabolic work was employed in these tests.

The capacity of the spirometer was not sufficient to hold a subject's exhalation for an entire test. It was only used as a pressure equalizing and mixing chamber into which the subject exhaled, and from which his exhalation was drawn at uniform rate through the meter by a pump. The rate of flow was controlled by a main valve and a bypass valve, so

that the spirometer bell floated slightly above or below the starting point as the rate of exhalation increased or decreased. At the close of the sampling period the bell was brought to the starting position and pressure, before the time and meter reading were recorded. A continuous sample of exhaled breath was collected at uniform rate from the gas stream passing to the meter in a mercury seal sampling tube.

As it was not comfortable for a man to breathe continuously into the apparatus, the exhalation of each subject was measured and sampled over three ½-hour periods near the beginning, middle, and end of the test period. The first ½-hour period began about eight minutes after the first weighing or about 20 minutes after the beginning of the test period. In order to flush the measuring and sampling apparatus and to insure that the subject had become accustomed to wearing the mask, he was required to breathe through the apparatus 8 minutes before the ½-hour sampling period began. A sample was collected at uniform rate in a mercury displacement sampling tube over the entire time of the three ½-hour periods. This was taken as a representative sample for the entire test and analyzed.

During each test at least three short time or grab samples, one during each half-hour period, were analyzed in order to indicate how nearly the total sample represented average conditions, also to indicate if the rate of metabolism changed during the test. Very little variation in metabolic rate was observed during the 4 hours of any test in moderate atmospheric conditions; such variation as was observed bore no apparent relation to test conditions, so that the long time samples were taken as a true measure of metabolism for the entire period.

Analyses of both representative and grab samples for carbon dioxide and oxygen were made on a modified Haldane gas apparatus. (Burrell and Seibert, 1926.) With this apparatus an accuracy in analysis of 0.02 per cent was obtained.

The effective temperature index. The effective temperature index is used to a great extent in analyzing the data presented. This index was introduced by Houghten and Yagloglou (1923), McConnell and Houghten (1923), Houghten and Yagloglou (1924), Yaglou and Miller (1925) and Houghten, Teague, and Miller (1926) as a result of their investigations at the Research Laboratory of the American Society of Heating and Ventilating Engineers of the relative effects of varying degrees of temperature, humidity, and air motion on human subjects. These results are summarized by Houghten and Teague (1928).

The chart, figure 2, is useful in any thermodynamic consideration of air, including water vapor or in a consideration of a person's feeling of warmth. In it, the dry-bulb temperature of the air is plotted as abscissae and grams of moisture per kilogram of dry air as ordinates. The maximum moisture which air can hold at various temperatures gives the saturation or 100 per cent humidity curve. Relative humidities

ranging from zero to 100 per cent are given by a series of similar curves. The wet-bulb temperatures as determined by a sling psychrometer are given by a series of nearly parallel oblique lines intersecting the dry-bulb temperature lines of the same degrees at the saturation curve. This much of the chart was developed by W. H.

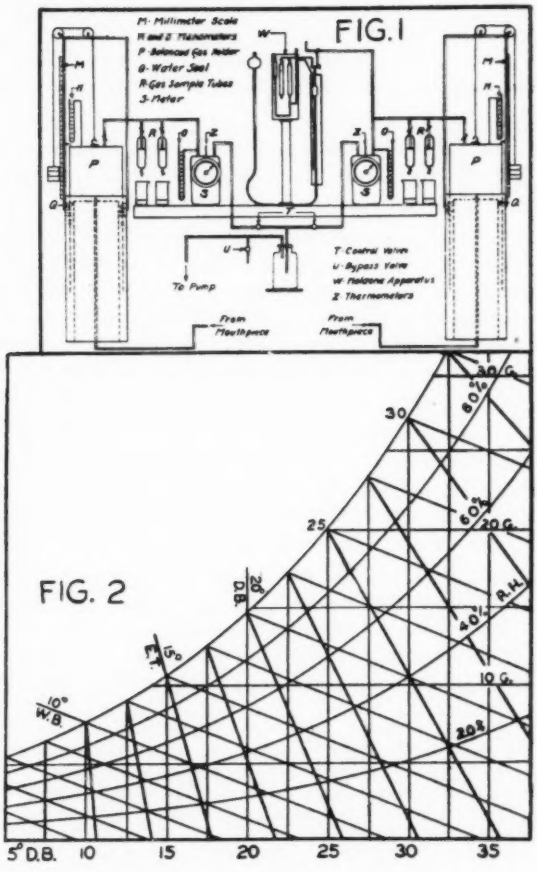

Fig. 1. Diagram of apparatus used for measuring, sampling, and analyzing exhaled breath.

Fig. 2. Psychrometric chart with effective temperature index for persons normally clothed and slightly active in still air.

Carrier (1911) and on it the equal comfort lines as determined by the Laboratory are superimposed giving the series of lines parallel to the dry-bulb temperature lines at a little above 7.2°C. (45°F.) and gradually approaching the same slope as the wet-bulb lines for high temperatures.

The slope of these effective temperature lines was experimentally determined by comparing the relative feeling of warmth experienced in two adjoining rooms in which the temperature, humidity, and motion of the air were controlled. The accuracy of the lines was later checked by the physiological reactions experienced by persons subjected to these conditions for several hours (McConnell and Houghten, 1923). A person normally clothed and at rest in still air will experience the same comfort or discomfort as regards feeling of warmth for all atmospheric conditions represented by any one of these lines. Air velocities move the effective temperature lines to the right so that a higher dry-bulb temperature is required for the same degree of warmth.

Test conditions. The data collected are for relative humidities of approximately 20, 45, 70, and 95 per cent, effective temperatures ranging from 6.7 to 37.8°C. and conditions of still air and air velocities of 1.19 and 1.96 meters per second (235 and 385 feet per minute). In fixing the limits of the effective temperature range it was desired to obtain data over the range met with in heating and ventilating practice. In order to establish the curves over this range, limits somewhat beyond those dictated by the needs of such practice were chosen. The upper practical limit was fixed at 32.2°C. effective temperature; the lower limit chosen was 6.7°C. effective temperature or the lowest which could be endured with the perscribed clothing without undue discomfort. Beyond 32.2°C. effective temperature, four tests were made in order to check the tendency of the curves at higher temperatures. These data collected above 32.2°C., give points that show a very definite tendency for the curves, but they may be slightly in error due to the necessarily shorter duration of the test, the inability of the body to adjust itself quickly to abnormal conditions, the excessive rises in body temperature, the excessive perspiration and the general effect of the hot conditions on the subjects and observers.

EXPERIMENTAL RESULTS. *Total heat production and loss.* The heat production in calories per square meter of body surface per hour, plotted

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Fig. 3. Heat production, all subjects.

Fig. 4. Total heat loss, all subjects.

Fig. 5. Average total heat loss for each of seven subjects.

Fig. 6. Heat loss by radiation and convection combined.

Fig. 7. Heat loss by evaporation.

In figures 3, 4, 6, and 7, curves *A* and *x* points for 20 per cent relative humidity; curves *B* and (square) points for 45 per cent; curves *C* and (triangle) points for 70 per cent; curves *D* and (circle) points for 95 per cent; curves *E*, average for all humidities; curve *F*, sensible heat loss from respiratory tract; curves *G* and *H*, latent heat loss from respiratory tract at 20 and 95 per cent relative humidity.

Figures 8 to 10, variation in heat loss at constant effective temperatures of 18.3, 23.9 and 29.4°C. respectively. Curves *A*, total heat loss; *B*, radiation and convection loss and *C*, evaporation loss.

All figures 3 to 10 for still air. All ordinate units, heat exchange in calories per square meter per hour. All abscissa units, air temperatures in degrees C. effective or dry bulb as indicated E. T. or D. B.

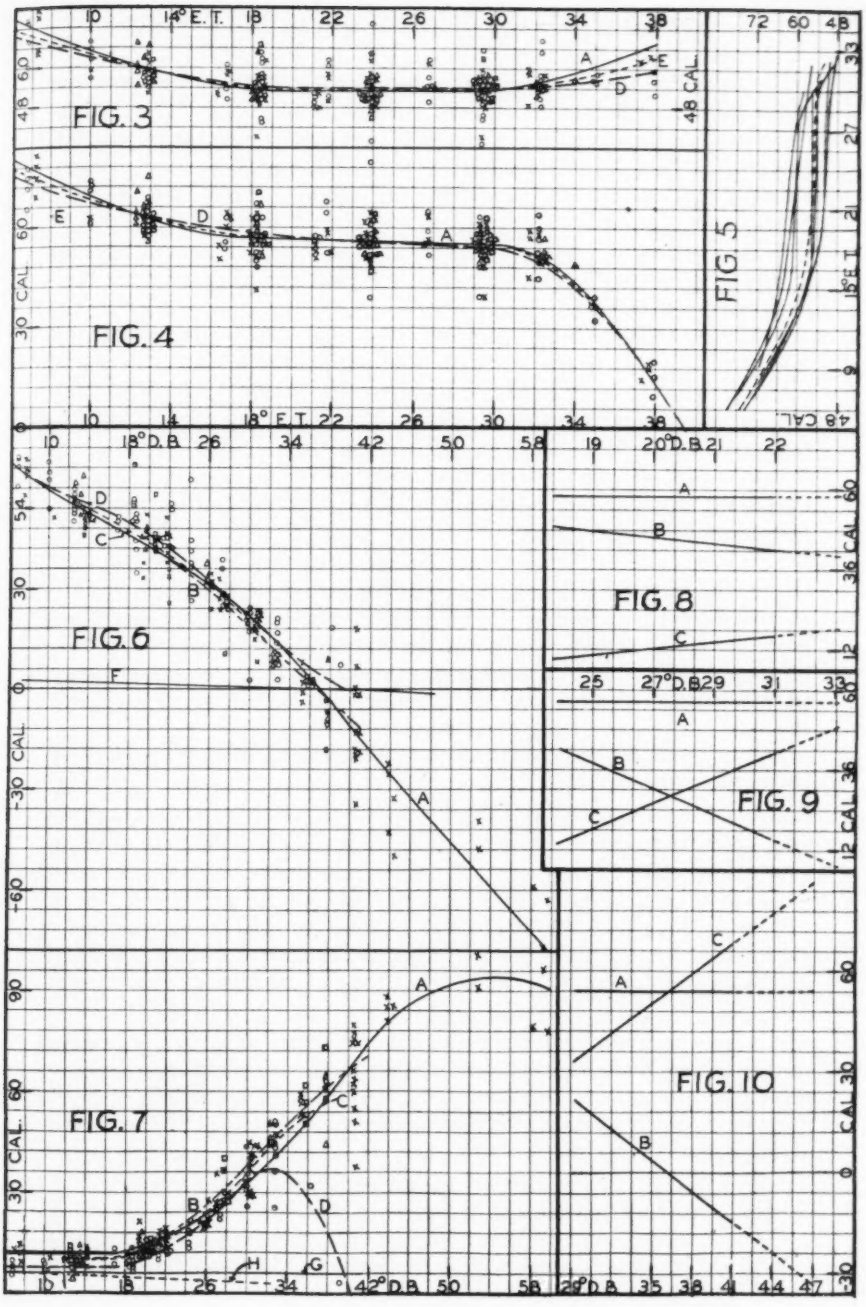
FIG. 3
FIG. 4
FIG. 5
FIG. 6
FIG. 7
FIG. 8
FIG. 9
FIG. 10

against effective temperature is given in figure 3. Body surface area was taken from the height-weight chart, according to Du Bois (1916). The various symbols distinguish the results of tests at different relative humidities. Curves are drawn for results of tests at 20 and 95 per cent relative humidity and also an average curve for results of tests at all relative humidities.

Total heat loss in calories per square meter of body surface per hour for all the subjects at all relative humidities is plotted against effective temperature in figure 4.

A positive increase in heat production at high and low temperatures is indicated, this is in agreement with other authorities cited. The rate of heat loss from the body is greater than the rate of heat production at low temperatures where one experiences a feeling of chill or where the body temperature falls below normal. In most of the tests here reported for temperatures below 30°C. the body temperature of the subjects fell slightly during the test period probably due to decrease in physical activity upon entering the chamber.

The body loses control of heat dissipation at high temperatures, and the rate of heat loss falls rapidly behind the rate of heat production until at an effective temperature approximately equal to body temperature heat loss becomes zero. That this is a little above normal body temperature or nearly 39.4°C. effective temperature may be accounted for by the fact that in this hot atmosphere the body temperature soon rises above normal.

Heat loss curves for each of the seven subjects and also the average curve for all subjects are given in figure 5. The variation in the individual curves may be accounted for by the variation in activity and diet, and the application of the Du Bois surface area chart to men of different physical proportions.

In the present investigation a minimum heat production and loss of 53.4 calories per square meter per hour is indicated as being practically constant within the temperature range, 19.3 to 28.7°C. Most metabolic determinations reported by other investigators have probably been made within this range, and we may compare the value of 53.4 here reported with values obtained by other authorities as given in table 2. In making this comparison, one should keep in mind the activity of the subjects in this investigation.

These figures, from a variety of sources, show that food and muscular exertion play a great part in the rate of heat production; also, the figures obtained in this investigation are in fair agreement with those found by other authorities when activity and diet are considered.

Heat loss by evaporation and by radiation and convection. Combined heat loss by radiation and convection for various relative humidities plotted

against dry bulb temperature is shown in figure 6. As would be expected from the physical law governing heat loss by radiation and convection, these curves are practically independent of relative humidity, or heat loss by radiation and convection combined, is a function of the difference between the temperature of the body and its environment. As the temperature of the body remains constant, it is a function of the temperature of that environment.

TABLE 2

Heat production and loss as given by various authorities for different conditions of activity and diet, expressed as Cal. per sq. m. per hour

AUTHORITY	CONDITIONS	CALORIES
DuBois (1927b)	Sitting indoors, dressing, eating, etc.	45.4–59.3
Macleod (1926)	Writing at desk	51.4
Hill (1919b)	Sitting indoors (16.6°C. E. T.)	57.1
Hill and Campbell (1922)	Sitting quietly with food (16°C. E. T.)	54.2
Atwater and Benedict (1903)	Indoor routine dressing, care of food, etc. — 7 a.m.–7 p.m. (most active hours)	57.5
	Indoor routine dressing, care of food, etc. — 7 p.m.–1 a.m. (leisure hours)	53.4
	Indoor routine dressing, care of food, etc. — 1 a.m.–7 a.m. (sleeping hours)	36.9
Benedict and Milner (1907)	Rest—very slight activity, carbohydrate diet	52.8
Benedict and Carpenter (1910)	At rest—sitting up	54.5
	At rest—standing	63.9
	Very severe muscular exercise	355.8
Benedict and Murchauser (1915)	Sitting without food	38.8
	Sitting after light meal	43.4
	Sitting after heavy meal	47.6
Lusk (1918)	Resting	42.5
Douglas, Haldane, Henderson, and Schneider (1913)	Resting in bed	38.0
	Rest standing	52.8
	Walking 2 miles per hour	104.2
McConnell, Yaglou, and Fulton (1925)	Basal (24°C. E. T.)	36.0
	Sitting at rest with food (24°C. E. T.)	40.0

Figure 7 gives the heat loss by evaporation or latent heat loss for various relative humidities plotted against dry-bulb temperature. Up to various temperatures, depending upon the relative humidity, heat loss by evaporation seems also to be a function of dry-bulb temperature. This would not be predicted for heat loss by evaporation if unlimited moisture were available, in which event evaporation would depend on both dry-bulb temperature and relative humidity. Through the heat-regulating center of the body the perspiration available is apparently so controlled that

evaporation, within that range of temperatures where the body maintains equilibrium, is a function of dry-bulb temperature and complementary to heat loss by radiation and convection.

As the dry-bulb temperature of the air rises and heat loss by radiation and convection decreases, the activity of the sweat glands is increased, resulting in greater evaporaton until a point is reached where the differences between the dew point temperature of the air and the surface temperature of the body (which may be taken as a measure of the affinity of the air for moisture) has decreased to such a value that evaporation falls off rapidly although one is entirely wet with perspiration. Since for constant dry-bulb temperature the dew point temperature increases with increase in relative humidity, the point where heat loss by evaporation ceases to be a function of dry-bulb temperature varies inversely with the percentage of relative humidity. For 95 per cent relative humidity, heat loss by evaporation begins to fall off at a little above 30°C., while at 70, 45 and 20 per cent heat loss by evaporation begins to fall off at approximately 35, 40, and 48°C.

For any given relative humidity the point where evaporation ceases to increase with increased dry-bulb the body fails to maintain temperature equilibrium and life becomes impossible for any considerable period.

The sensible and latent heat loss from the respiratory tract shown in figures 6 and 7 were calculated from the weight, temperature, and moisture content of the inspired air and the temperature and degree of saturation of the exhaled breath as determined in this investigation. Sensible heat

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Figs. 11 and 12, relation of heat loss to effective temperature at 20 and 95 per cent relative humidity respectively; curves *A*, total loss; curves *B*, radiation and convection loss, and curves *C*, evaporation loss.

Figs. 13 and 14, relation of total heat loss in still and moving air to effective and dry bulb temperature respectively.

Fig. 15, relation of weight loss in grams per square meter per hour (left) and heat loss, by evaporation (right) to dry bulb temperature in still and moving air.

Fig. 16, relation of heat loss by radiation and convection in still and moving air to dry bulb temperature.

In figures 13 to 16 curves *A* are for still air and 20 per cent relative humidity; curves *B* for air velocities of 1.19 m. per second, and 20 per cent relative humidity; curves *C* for air velocities of 1.96 m. per second and 20 per cent relative humidity; curves *D* for still air and 95 per cent relative humidity; curves *E* for air velocities of 1.19 m. per minute and 95 per cent relative humidity; curves *F* for air velocities of 1.96 m. per minute and 95 per cent relative humidity; curves *G* and *H*, loss from respiratory tract at 20 per cent and 95 per cent relative humidity; curve *I*, weight loss grams per square meter per hour, due to gaseous exchange.

Fig. 17 relation between rate of heat production and body temperature.

In figure 15, left ordinate units, weight loss in grams per square meter per hour; all other ordinate units, figures 13 to 17, heat exchange in calories per square meter per hour; abscissa, figure 17, body temperature; abscissa, figures 13 to 17, air temperature degrees C. effective, or dry bulb as indicated E. T. or D. B.

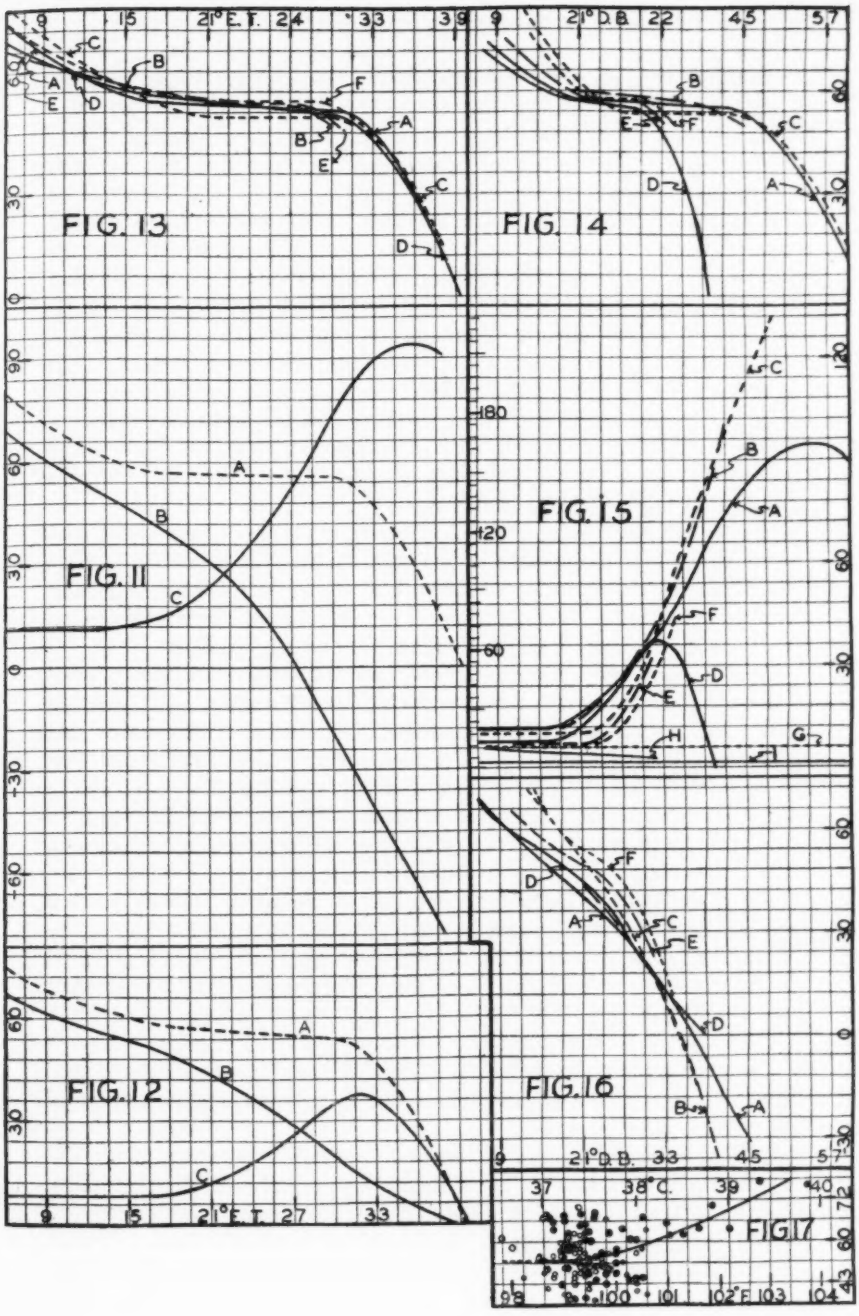
FIG. 13
FIG. 14
FIG. 11
FIG. 15
FIG. 12
FIG. 16
FIG. 17

loss from the respiratory tract is always a very insignificant figure. Latent heat loss from the respiratory tract is a considerable percentage of the total latent heat loss for temperatures below 20°C., but decreases slightly for higher temperatures while the total latent heat loss increases rapidly.

Variation in heat loss by evaporation and radiation and convection at constant effective temperature. The variation in heat loss by radiation and convection and by evaporation with dry-bulb temperature at constant effective temperatures of 18.3, 23.9 and 29.4°C. is shown in figures 8, 9, and 10, respectively. Total heat loss is constant within the limits of experimental error for any effective temperature, whereas evaporation loss and the combined loss by radiation and convection both vary with dry-bulb but so as to be complements of each other in making up the total loss. For any effective temperature, evaporation loss increases and radiation and convection loss decreases with ascending temperature. The charts also serve to show the variation of dry-bulb temperatures with relative humidity for these effective temperatures.

Variation in heat loss by evaporation and by radiation and convection with relative humidity. Heat losses by evaporation and the combined loss by radiation and convection for all subjects at 20 per cent relative humidity are given in figure 11. The total heat losscurve for all subjects is also given. Similar data are given for 95 per cent relative humidity in figure 12.

Radiation and convection combined, and evaporation are functions of effective temperature with constant relative humidity but not with variable relative humidity as shown by comparison of the curves at 20 per cent and 95 per cent relative humidity. For temperatures up to 16°C. radiation and convection are the predominating avenues of heat loss. At 16°C., with 95 per cent relative humidity, radiation and convection account for 88 per cent of the total loss, whereas for the same temperature and 20 per cent relative humidity these losses account for 78 per cent of the total.

It is of interest to note that heat loss by radiation and convection decreases as the dry-bulb temperature of the air approaches that of the body and reaches zero at 38.7°C. effective temperature with 95 per cent relative humidity and at 27.6°C. effective temperature with 20 per cent, these conditions giving 39.4°C. and 37.1°C. dry-bulb respectively. It should be observed that the former condition, 38.7°C. effective temperature, is more severe than the latter, 27.6°C. effective temperature and that body temperature rises considerably above 37, approaching an average of 39.4° during the test period.

While this decrease in radiation and convection is taking place the evaporation loss increases so as to maintain the total heat loss practically constant until an effective temperature of 32°C. is reached, when with 95 per cent relative humidity the evaporation loss reaches a maximum of 74 per cent of the total, while with 20 per cent relative humidity the evapora-

tion loss reaches 163 per cent of the total at this temperature and continues to increase until a maximum of 285 per cent of the total is reached at 35.6°C. effective temperature. The loss by evaporation in excess of the total heat loss is compensated for by an equal heat gain by radiation and convection shown by negative values on this curve.

Effect of air velocity on heat exchange between the body and air. Total heat loss for 20 and 95 per cent relative humidity in still air and in air velocities of 1.19 and 1.96 meters per second (235 and 385 feet per minute) are plotted against effective temperature in figure 13. It will be observed that there is little separation of the curves except at low temperatures where separation may be due to experimental error, indicating that total heat loss is a function of effective temperature in either still or moving air.

That total heat loss is not a function of dry-bulb temperature for still and moving air is clearly indicated by an examination of figure 14 where the same data shown in figure 13 are plotted against dry-bulb temperature.

Heat loss by evaporation and by radiation and convection combined for 20 per cent and 95 per cent relative humidity in still and moving air is given in figures 15 and 16. As one should expect from our knowledge of the physical laws of heat transfer, combined heat loss by radiation and convection increases for the same dry-bulb temperature with air velocity. For those conditions where heat is transmitted from the environment to the body by radiation and convection, heat gain is also increased with air velocity.

This is borne out by Lefevre (1911) who gives results for persons clothed showing an increased heat loss by radiation and convection with air velocity for all dry-bulb temperatures below 30°C. The greater the velocity the greater the loss.

With unlimited moisture available one would expect heat loss by evaporation also to increase with air velocity for the same dry-bulb temperature. This is not the case, however, with heat loss by evaporation from the human body. Figure 15 shows that heat loss by evaporation is minimal below 17°C. for still air and below 22°C. for the air velocities studied. Evaporation loss decreases with air velocity for temperatures lower than about 32°C., indicating a control of perspiration available for evaporation so that as heat loss by radiation and convection increases due to air motion, less perspiration is made available for evaporation and this loss decreases.

Weight loss by evaporation in grams per square meter of body surface per hour is given by the scale on the left side of figure 15. The values for evaporation loss either in grams per hour or in heat loss by evaporation, substantiate in many instances the findings of other investigators. Hill (1919c) states that Wolpert has determined that the evaporation of water from a resting man is minimal from 18°C. to 20°C. in still air and at 27°C.

in moving air; at temperatures below 20°C. wind increases this loss about 5 per cent; for temperatures from 20 to 35°C. wind lowers the evaporation by 33 to 50 per cent; at temperatures of 36°C. and over, wind doubles or more than doubles the loss in still air. The loss by evaporation from the lungs of a sedentary man at 20°C. is estimated by Hill (1919d) as 400 grams for 24 hours (9.2 grams per square meter per hour for an assumed body surface area of 1.81 square meters).

He further gives data from Rubner which, when translated into grams per square meter per hour for the body surface area given above, are as follows:

DEG. C.	IN DRY AIR		IN MOIST AIR	
	Lungs	Skin	Lungs	Skin
	grams	*grams*	*grams*	*grams*
15	9.3	5.2	5.0	
20	9.4	20.5	6.5	2.0
25	10.2	31.5	6.0	7.2

Benedict (1927) in a review of the literature on insensible perspiration concludes from the findings of others that the insensible loss will amount to 15 to 60 grams per hour (8.3 to 33.2 grams per square meter per hour for an assumed body surface area of 1.81 square meters), and in general the total loss is proportional to the size of the individual and metabolic rate for any condition of health and activity. The effect of clothing, air current and surrounding temperature is small. Only when the temperature exceeds 26°C. is there an increase which is generally associated with sweating. The loss decreases if the body becomes cold. It is also shown that the insensible loss varies with the heat production.

In addition to his review of the literature, Benedict gives data for six presumably healthy subjects as the result of his investigation. His results expressed as averages are: Body weight, 66.5 kgm.; total insensible loss, 26.6 grams per hour; skin loss, 13.2 grams per hour (14.7 and 7.3 grams per square meter per hour based on assumed body area of 1.81 square meters). The subjects were lying down, clothed and covered in most cases, with a light woolen blanket in a room at 20°C. He states that the losses are practically the same for persons clothed without circulation, unclothed without circulation, and unclothed with circulation.

Benedict's conclusion that moisture evaporated from the body is proportional to the metabolic rate for any condition of health or muscular activity, is substantiated by the finding of the study here reported in so far as excessive metabolic rates were encountered and is in keeping with the theoretical deduction, reached earlier in this report, that heat loss by

radiation and convection is practially independent of body control and a function of atmospheric conditions and that total heat loss in controlled and temperature equilibrium is maintained largely by controlling perspiration available for evaporation. Benedict's conclusion must, however, in the light of the findings presented in figure 15 be limited in its rigid application to conditions of constant dry-bulb temperatures, relative humidity, and air motion. Less rigidly it will apply for dry-bulb temperatures below 18 or 20°C. for which conditions loss by evaporation is not greatly affected by temperature, humidity or air motion. Above 20 or 25°C. it will not apply, however, without considerable error.

Metabolic rate as a function of body temperature. The relation of heat production to body temperature is given in figure 17. There is a distinct rise in the metabolic rate with increase of rectal temperature above 37.3°C.

TABLE 3

Condition of sensible perspiration for various atmospheric conditions

DEGREE OF PERSPIRATION	EFFECTIVE TEMPERATURE AT	
	95% R.H.	20% R.H.
Forehead clammy	22.8	23.9
Body clammy	22.8	23.9
Body damp	26.2	27.2
Beads on forehead	26.7	30.6
Body wet	29.2	30.3
Perspiration on forehead runs and drips	31.1	34.4
Perspiration runs down body	31.4	32.2

40 per cent of subjects registered degree of perspiration equal to or greater than indicated.

(99°F.). This indicates a failure of physiological control of heat production where such control is most needed and assertion of the purely chemical laws of combustion, according to which uncontrolled chemical reactions in general and particularly oxidation reactions increase in rate with temperature. That this law holds for many biological reactions is indicated by Du Bois (1927c) who quotes from a number of authorities, including Van't Hoff, the formulator of the law.

The dark shaded points are for short time metabolism determinations within a long time exposure to a constant high temperature atmosphere. These tests indicate that the metabolic rate at high temperature is not a direct function of atmospheric temperature but rather a function of body temperature. Since with exposure for a fixed period in atmospheres of different temperatures, body temperatures will depend on the effective tem-

perature of the atmosphere, metabolic rate may under such conditions be an indirect function of the effective temperature.

Sensible perspiration. Table 3 gives the degree of sensible perspiration for different conditions of temperature and humidity. Perspiration loss changes from insensible to sensible at 22.8 and 23.9°C. effective temperature for 20 and 95 per cent relative humidity respectively. Above this temperature the quantity steadily increases until at from 31 to 34°C. effective temperature, the flow is excessive from the entire body.

SUMMARY

A series of 267 tests was made on seven normal male subjects at effective temperatures of from 6.7 to 37.8°C., humidities of 20, 45, 70, and 95 per cent and in still air and air velocities of 1.19 and 1.96 meters per second (235 and 385 ft. per minute). The subjects had previously partaken of their regular diet, were normally clothed and seated a great part of the time, but were not restrained from normal movement of limbs and body. A minimum of 53.4 calories per square meter per hour was found for the temperature range 19.3 to 28.7°C., a value in close agreement with results of other investigators for men under like conditions of activity and diet. For higher and lower temperatures there is an increase in the metabolic rate.

The effective temperature index of sense of warmth and physiological reactions for varying temperatures, humidities, and air motion is defined and heat production within and total heat loss from the body are shown to be functions of this index for both still and moving air.

Total heat loss from the body is divided into loss by evaporation and combined loss by radiation and convection, both of which are shown to be functions of dry-bulb temperature; the former increases and the latter decreases as the dry-bulb temperature scale is ascended.

Change from insensible to sensible perspiration takes place at 22.8 and 23.9°C. effective temperature with 95 and 20 per cent relative humidity respectively. The change in degree of perspiration with temperature and humidity is shown.

The human body maintains temperature equilibrium in atmospheres of varying temperature and humidity by controlling heat production at low atmospheric temperatures and by controlling evaporation by control of available perspiration at high temperatures.

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METABOLISM FOLLOWING ANOXEMIA

I. Oxygen Consumption and Blood Lactates after Experimentally Induced Exercise

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The phenomenon of "recovery oxidation" (A. V. Hill, 1926) presents some interesting problems. For an hour or more after the cessation of exercise the oxidative metabolism of the organism is pitched on a higher level than before the exercise. Evidently some stimulus to oxidative metabolism is brought into operation in consequence of exercise, the effect of which subsides slowly. The agent to which the rôle of stimulant has been assigned is lactic acid or, more strictly, the lactate ions (A. V. Hill, 1926, and others). There are obvious reasons for assigning the rôle to lactates. For one, they are the chief easily demonstrable compounds other than carbon dioxide which are both thrown into the blood in quantity during exercise and persist therein for a considerable time thereafter. Furthermore, there is abundant inferential basis for the assignment, inasmuch as the accumulation of lactates in tissues and blood is a consequence of inadequate oxygenation of the active muscles, and one may logically consider, as A. V. Hill has done, that the excess oxidative metabolism functions as the chief means of clearing the tissues and blood of the lactates thus accumulated.

An important point in the theory as developed by Hill is that the amount of excess oxygen consumed is taken to be a definite function of the quantity of lactate removed during the recovery process. Recovery oxidation is pictured, therefore, as due to the calorigenic action of lactate ions and as operating quantitatively to remove excess lactates from the exercised organism.

There is evidence from several sources in favor of the view that lactates may display calorigenic action (Lusk, 1917; Meyerhof, Lohman and Meier, 1925). The main experimental basis for the view that the recovery oxidation is quantitatively adjusted to the amount of lactate disposed of is in the work of A. V. Hill, Meyerhof and others on the recovery process in excised frogs' muscles (see A. V. Hill, loc. cit. for citations). Determinations of the recovery heat production on the one hand and of the

quantity of lactate disappearing on the other gave values corresponding to the oxidation of 1 molecule of lactic acid for each 5.2 molecules of the acid disappearing. Other evidence indicates that the unoxidized portion is resynthesized to glycogen.

In a comparatively limited number of experiments on men Hill and associates (A. V. Hill, loc. cit.) have obtained figures which they interpret as showing the above ratio of lactate oxidized (as computed from the recovery oxygen consumption) to lactate disappearing to apply to intact higher organisms. This identity in the ratio constitutes the main experimental basis for carrying over to man and the higher animals the view that recovery oxidation functions quantitatively to remove the accumulated lactates of exercise.

The matter is of prime importance, not only to a proper understanding of muscle metabolism, but to the elucidation of oxidation-reduction phenomena, as they occur in living tissues, hence continued investigation seems to us to be in order. That a similar idea has occurred to other workers is evidenced by the recent publication by H. A. Abramson and P. Eggleton (1927) of a series of studies of the utilization by the organism of sodium-*r*-lactate, intravenously injected. They conclude, as one result of their investigation, that the lactate ion is not the factor responsible for excess recovery oxidation.

During 1926 we performed a series of experiments similar to those reported by Abramson and Eggleton, with results corresponding to theirs. We employed massive doses of sodium-*r*-lactate, calculated to introduce amounts of lactate equivalent per kilo of body weight to those stated by A. V. Hill to be present in the body at the end of a bout of severe exercise. The experimental animals (dogs, anesthetized with chloretone) endured the injections well; no serious disturbances in blood pressure or lung ventilation were observed. We were unable to demonstrate increases in oxygen consumption over the initial values, thus confirming the findings of Abramson and Eggleton.

We were not satisfied with the above procedure, however, in that it does not at all resemble the normal process by which the tissues and fluids of the body become charged with lactates. Consequently we undertook to bring about lactate production directly in the tissues of experimental animals. The principle to be followed was obvious; it having long been known that in tissues subjected to anoxemia the energy metabolism follows a course resulting in accumulation of lactates (presumably *d*-lactates exclusively). Our task, then, was to subject experimental animals to anoxemia and to study the course of oxygen consumption in comparison with variations in the concentration of the excess blood lactates induced by the anoxemia.

Anoxemia due to exercise. Our first method of inducing anoxemia was

to subject our animals to severe exercise. It was particularly important that this be done in order to show whether or not typical "oxygen debt" can be induced in animals rendered unconscious by drugs or by brain-stem transections under the usual conditions of physiological experimentation; i.e., conditions permitting of continuous blood-pressure tracings and such additional operative procedures as the study might require. So far as we are aware this point had not been investigated prior to the beginning of our work (Martin, Field and Hall, 1928).

In our first experiments of this group dogs anesthetized with amytal (about 55 mgm. per kilo, intraperitoneally) were used (designated A in tables). During the progress of the work a paper by Long (1928) came to our attention, in which amytal anesthesia was reported to interfere with normal resynthesis of glycogen in muscles following depletion by exercise. We consequently carried out a second series in which brain-stem transection under transient ether anesthesia was employed as the method of abolishing consciousness (designated G in tables).

Artificial exercise was induced by rhythmic tetanization of the spinal cord, at the rate of 96 brief tetanizations per minute. The stimulating electrodes were implanted beneath the skin in the mid-cervical and sacral regions along the dorsal mid line. The exercise was continued for 15 minutes. The arterial pressure (registered from a carotid artery) and the breathing were watched continually, and the stimuli were made about as strong as would be endured without undue disturbance of the cardio-vascular and respiratory mechanisms. We found that exercise sufficiently severe for our purpose could be induced thus.

A continuous record of oxygen consumption was made by means of a Sanborn "grafic" type of Benedict closed-circuit metabolism apparatus. In this apparatus, oxygen in high concentration is breathed from a spirometer bell, and the carbon dioxide and water vapor given off by the organism are absorbed by a specially prepared soda lime. Our only modification was to substitute a long paper, carried in part by an extra drum, for the eight-minute paper ordinarily used in determinations on human subjects. A large-bore cannula was tied in the trachea of the experimental animal and connected by a short and wide rubber tube with the opening to which the mouth-piece is regularly attached. The oxygen was kept in rapid circulation by the blower that is included as part of the Sanborn instrument. Corrections for bell temperature were made when indicated, according to the table provided by the makers.

By means of a mercury thermometer thrust well into the rectum, rectal temperatures were noted at frequent intervals throughout the experiments. The animals were kept under observation for 90 minutes following the end of exercise, except in a few cases in which the arterial pressure fell unduly, bringing the experiments to an end earlier. The mean rectal

TABLE 1

Data on oxygen consumption, arterial pressure and rectal temperature

A, amytal; G, brain-stem transection

EXPERIMENT NUMBER	BODY WEIGHT		PRE-EXERCISE (BASAL)	EXERCISE	POST-EXERCISE; PERIODS 15 MINUTES EACH					
					1	2	3	4	5	6
	kgm.									
A 6	11.7	O_2 per min. cc.	55	250	77	77	78	75	71	69
		Rectal temp.	*39.4*	*39.8*	*40.1*	*40*	*40*	*39.7*	*39.4*	*39.1*
		Mean arter. pr. mm. Hg	*140*	*120*	*145*	*142*	*142*	*138*	*132*	*130*
		Calc. basal O_2			59	58	58	57	55	53
		Excess O_2 per min.			**18**	**19**	**20**	**18**	**16**	**16**
8	8.1	O_2 per min. cc.	39	113	50	47	51	50	51	51
		Rectal temp.	*36.4*	*36.7*	*37*	*37*	*37*	*37*	*37.3*	*37.5*
		Mean arter. pr. mm. Hg	*95*	*90*	*100*	*97*	*85*	*75*	*77*	*77*
		Calc. basal O_2			41	41	41	41	42	43
		Excess O_2 per min.			**9**	**6**	**10**	**9**	**9**	**8**
9	16.4	O_2 per min. cc.	71	658	150	130	132	115	116	114
		Rectal temp.	*36.5*	*38*	*39.4*	*39.2*	*39.1*	*39*	*39*	*39*
		Mean arter. pr. mm. Hg	*115*	*150*	*170*	*155*	*145*	*145*	*142*	*140*
		Calc. basal O_2			92	90	89	88	88	88
		Excess O_2 per min.			**58**	**40**	**43**	**27**	**28**	**26**
10	16.6	O_2 per min. cc.	89	570	166	128	117	108	101	101
		Rectal temp.	*36.4*	*37.8*	*38.8*	*38.7*	*38.6*	*38.5*	*38.5*	*38.5*
		Mean arter. pr. mm. Hg	*158*	*120*	*142*	*142*	*142*	*142*	*142*	*142*
		Calc. basal O_2			110	109	108	107	107	107
		Excess O_2 per min.			**56**	**19**	**9**	**1**		

11	8.5	O_2 per min. cc.		55		388		93		83		76		73		70		66
		Rectal temp.	*38.6*		*40.3*		*41.4*		*40.9*		*40.4*		*40.1*		*40*		*40*	
		Mean arter. pr. mm. Hg	*110*		*110*		*88*		*112*		*120*		*120*		*95*		*88*	
		Calc. basal O_2						70		68		65		63		63		63
		Excess O_2 per min.						**23**		**15**		**11**		**10**		**7**		**3**
12	15.5	O_2 per min. cc.		71		260		108		100		97		97		98		98
		Rectal temp.	*38.4*		*38.8*		*39*		*38.9*		*38.8*		*38.8*		*38.8*		*38.9*	
		Mean arter. pr. mm. Hg	*100*		*70*		*72*		*85*		*95*		*100*		*105*		*110*	
		Calc. basal O_2						75		74		74		74		74		74
		Excess O_2 per min.						**33**		**26**		**23**		**23**		**24**		**24**
14	13.6	O_2 per min. cc.		77		200		104		90		92		84				
		Rectal temp.	*37.7*		*38.7*		*39*		*39.2*		*39.7*		*40*					
		Mean arter. pr. mm. Hg	*80*		*65*		*45*		*40*		*35*		*30*					
		Calc. basal O_2						87		89		92		95				
		Excess O_2 per min.						**17**		**1**		**0**						
15	13.6	O_2 per min. cc.		58		256		89		75		72		71		73		72
		Rectal temp.	*38*		*38.8*		*39.5*		*39.4*		*39.2*		*39*		*38.8*		*38.7*	
		Mean arter. pr. mm. Hg	*95*		*80*		*95*		*100*		*105*		*100*		*100*		*100*	
		Calc. basal O_2						67		66		65		64		63		62
		Excess O_2 per min.						**22**		**9**		**7**		**7**		**10**		**10**
16	10.5	O_2 per min. cc.		61		100		71		68		73		77		77		76
		Rectal temp.	*38.4*		*38.6*		*38.7*		*38.7*		*38.9*		*39*		*39.3*		*39.4*	
		Mean arter. pr. mm. Hg	*80*		*60*		*80*		*75*		*72*		*72*		*72*		*78*	
		Calc. basal O_2						63		63		64		65		66		67
		Excess O_2 per min.						**9**		**5**		**9**		**12**		**11**		**9**

TABLE 1—*Concluded*

EXPERIMENT NUMBER	BODY WEIGHT		PRE-EXERCISE (BASAL)	EXERCISE	POST-EXERCISE; PERIODS 15 MINUTES EACH					
					1	2	3	4	5	6
	kgm.									
17	12.8	O_2 per min. cc.	75	352	100	100	100	94	96	95
		Rectal temp.	*36.7*	*38.3*	*39.3*	*39*	*38.9*	*38.9*	*38.8*	*38.8*
		Mean arter. pr. mm. Hg	*120*	*110*	*125*	*135*	*140*	*135*	*138*	*135*
		Calc. basal O_2			94	92	92	92	91	91
		Excess O_2 per min.			**6**	**8**	**8**	**2**	**5**	**4**
G 1	12.0	O_2 per min. cc.	88	159	94	94	96	78		
		Rectal temp.	*37.8*	*38.1*	*38.5*	*38.5*	*38.5*	*38.5*		
		Mean arter. pr. mm. Hg	*150*	*120*	*80*	*72*	*60*	*45*		
		Calc. basal O_2			94	94	94	94		
		Excess O_2 per min.			**0**	**0**	**2**			
2	13.0	O_2 per min. cc.	127	675	181	162	158	155	152	150
		Rectal temp.	*38.4*	*40.5*	*41.8*	*41.5*	*41.4*	*41.3*	*41.2*	*41.2*
		Mean arter. pr. mm. Hg	*150*	*170*	*180*	*165*	*155*	*140*	*135*	*125*
		Calc. basal O_2			170	166	165	164	162	162
		Excess O_2 per min.			**11**					
3	20.0	O_2 per min. cc.	170	490	230	234	238	247	242	243
		Rectal temp.	*38.1*	*40.0*	*40.9*	*40.6*	*40.3*	*40.1*	*40.4*	*41.5*
		Mean arter. pr. mm. Hg	*142*	*165*	*165*	*150*	*130*	*100*	*78*	*60*
		Calc. basal O_2			218	212	208	204	209	228
		Excess O_2 per min.			**12**	**22**	**30**	**43**	**33**	**15**
4	16.4	O_2 per min. cc.	116	597	190	167	166	159	157	150
		Rectal temp.	*39.0*	*40.6*	*42.0*	*41.8*	*41.8*	*41.8*	*41.9*	*42.2*
		Mean arter. pr. mm. Hg	*108*	*140*	*155*	*142*	*130*	*120*	*105*	*100*
		Calc. basal O_2			151	148	148	148	150	153
		Excess O_2 per min.			**39**	**19**	**18**	**11**	**7**	

		Excess O_2 per min.					**39**		**19**		**18**		**11**		**7**		
6	11.8	O_2 per min. cc.		86		224		117		110		111		111		113	109
		Rectal temp.	*37.8*		*38.6*		*39.6*		*39.8*		*39.8*		*40.0*		*40.1*		*40.2*
		Mean arter. pr. mm. Hg	*80*		*95*		*88*		*85*		*80*		*60*		*60*		*67*
		Calc. basal O_2						101		103		103		105		106	107
		Excess O_2 per min.						**16**		**7**		**8**		**6**		**7**	**2**
7	16.0	O_2 per min. cc.		76		390		116		109		106		99		95	97
		Rectal temp.	*37.3*		*39.1*		*39.9*		*39.7*		*39.6*		*39.6*		*39.5*		*39.6*
		Mean arter. pr. mm. Hg	*145*		*140*		*162*		*155*		*155*		*153*		*140*		*130*
		Calc. basal O_2						96		94		93		93		93	93
		Excess O_2 per min.						**20**		**15**		**13**		**6**		**2**	**4**
8	8.9	O_2 per min. cc.		50		327		83		78		79		81		80	84
		Rectal temp.	*36.4*		*38.2*		*38.6*		*38.4*		*38.1*		*38.2*		*38.4*		*38.8*
		Mean arter. pr. mm. Hg	*75*		*120*		*70*		*80*		*75*		*85*		*80*		*66*
		Calc. basal O_2						61		60		58		59		60	62
		Excess O_2 per min.						**22**		**18**		**21**		**22**		**20**	**22**
9	8.7	O_2 per min. cc.		60		206		101		89		81		81		72	
		Rectal temp.	*37.7*		*39.2*		*40.6*		*40.7*		*40.8*		*40.7*		*40.7*		
		Mean arter. pr. mm. Hg	*110*		*150*		*135*		*110*		*80*		*85*		*60*		
		Calc. basal O_2						77		78		79		78		78	
		Excess O_2 per min.						**23**		**11**		**2**		**3**			
10	9.0	O_2 per min. cc.		69		146		103		83		82		81		85	95
		Rectal temp.	*39.2*		*39.9*		*40.3*		*40.6*		*40.7*		*40.9*		*41.0*		*41.2*
		Mean arter. pr. mm. Hg	*72*		*85*		*68*		*77*		*80*		*75*		*72*		*68*
		Calc. basal O_2						76		78		79		81		81	83
		Excess O_2 per min.						**27**		**5**		**3**		**0**		**4**	**12**

temperatures and mean arterial pressures for each 15-minute interval of each experiment are set down in table 1. It will be noted that only in experiment A 14 and during the last 15-minute period of experiment G 1 did the arterial pressure fall below 60 mm. Hg. Experiment A 14 is included in the series because both the oxygen consumption picture and the blood lactate picture (see tables) correspond in general with the pictures seen in other experiments in which arterial pressure was satisfactorily high.

Blood samples for lactate determinations were taken in the earlier experiments from the left femoral artery, directly distal to the branching of the deep femoral. Although we saw no indication that the circulation through the leg had been deleteriously affected by non-participation of the femoral beyond the point of cannulization we changed in later experiments to the carotid not connected with the blood-pressure manometer, thus leaving the significant part of the circulation intact.

Blood samples were taken just before and just after the exercise, and 15 minutes, 30 minutes, 60 minutes and 90 minutes after it was over. Proteins were precipitated by the method of Folin and Wu (1919) and carbohydrates by the method of Van Slyke (1917). Blood lactates were determined by the method of Friedmann, Cotonio and Shaffer (1927). Duplicate determinations were run on many of the samples, with as good agreement as the degree of refinement of the method indicates.

Table 1 gives the data on oxygen consumption. In the first horizontal column for each experiment are set down the mean consumption per minute just before exercise, during exercise, and during successive 15-minute periods till the end of the experiment. These figures show, as would be anticipated, a pronounced rise during the period of artificially induced exercise, followed by a more or less gradual return toward the pre-exercise level. In only one experiment (G 1) was that level reattained by the end of the experimental period. The persistently higher level of oxygen consumption is to be accounted for in part, although not altogether by the heightened body-temperature consequent upon the exercise. The basal metabolic rate is stated to increase about one per cent for each tenth degree Centigrade rise in body temperature (Du Bois, 1921). In accordance with this principle we computed for each post-exercise stage of each experiment the theoretical basal oxygen consumption. The calculations are set down in the fourth horizontal columns for the successive experiments. Subtracting the results from the observed oxygen consumptions gives figures for the excess oxygen consumption attributable to the exercise *per se*, and designated by A. V. Hill the "recovery" oxygen. These data are given in the fifth horizontal columns for the different experiments.

It will be seen that in slightly less than half of our experiments (8 out

of 19) even after making allowance for body temperature rise, a considerable excess oxygen consumption continued for as long as ninety minutes after the end of exercise, as a rule falling off relatively slightly after the first fifteen-minute post-exercise period. In the other experiments there was little or no excess late in the post-exercise period, although in all but three a well-marked excess was noted during the half-hour immediately following exercise. In view of the observations of Long cited above (p. 409) indicating a different course of intrinsic muscle

TABLE 2

Arterial blood lactates, mgm. per 100 cc.

A, amytal; G, brain-stem transection

EXPERIMENT	BEFORE EXERCISE	AT END OF EXERCISE	AFTER 15 MINUTES	AFTER 30 MINUTES	AFTER 60 MINUTES	AFTER 90 MINUTES
A 6	13.0	49.5		30.7	22.3	
8	21.8	27.7	22.8	22.8	23.1	
9	7.5	33.6	29.3	25.2	12.2	10.5
10	23.1	52.0	49.2	35.3	29.9	25.2
11	7.2	39.5	33.7	26.4	20.9	21.0
12	21.8	46.4	29.2	22.6	22.3	21.5
14	37.5	52.8	41.9	38.4		
15	27.8	71.2	55.0	43.3	34.8	26.8
16	20.8	30.8	35.9	27.7	25.8	25.2
17	10.3	35.2	27.5	20.0	16.2	14.1
G 1	27.7	39.7	29.8	25.2	18.1	
2	50.8	61.3	54.2	47.9	54.6	50.6
3	27.3	29.6	25.2	22.2	22.7	23.5
4	35.3	68.0	45.8	45.4	34.2	35.4
6	28.3	44.2	33.8	31.3	33.7	37.4
7	37.1	60.0	47.2	48.3	39.1	42.2
8	38.5	57.4	57.3	51.9	34.3	25.4
9	x*	x*	93.0	59.2	48.7	50.7
10	27.8	59.8	44.1	29.5	23.3	28.5

* Samples accidentally destroyed.

metabolism in animals anesthetized with amytal and animals subjected to brain-stem transection, it should be noted that in our experiments no consistent differences in the post-exercise oxygen consumption pictures were seen as between the two groups, although from Long's results one might have anticipated a larger "recovery" oxygen consumption in the animals of the latter group than in those of the former.

We turn now to the consideration of the changes in blood lactates consequent on the artificially induced exercise. These are set down in table 2. The following points appear to merit comment: Blood lactates increased

markedly (40 per cent to 450 per cent) in all the experiments except three. The lactate concentration was higher immediately after the exercise than at any subsequent analysis except in experiment A16 in which the blood sample drawn fifteen minutes later showed a higher lactate concentration than the sample drawn directly after the exercise. After the peak the lactate concentration fell toward the pre-exercise level in all the experiments. This level was attained or approached closely within 30 to 90 minutes in all the experiments except four of series A in which the initial lactate concentration was unusually low. In these the blood sample drawn at the end of the experiment still showed a considerably higher lactate content than the pre-exercise blood.

Discussion. According to the postulate of A. V. Hill mentioned earlier (p. 407) one would expect to find a fairly definite quantitative relationship between "recovery" oxygen-consumption and the concurrent disappearance of excess lactates due to exercise. We are obliged to confess our inability to find in these experiments satisfactory indication of such a relationship.

The data set down in columns 6, 7, and 9 of table 3 serve as the basis for this conclusion on our part. Column 6 gives the total excess oxygen for the entire post-exercise period, as calculated from the data in table 1 in the fifth horizontal columns for the several experiments. To obtain the figures in column 7 we followed the procedure of Hill, Long and Lupton (1924a), who found that after a bout of exercise in man the oxygen consumption had usually not returned to the pre-exercise level by the end of the rather long recovery period allowed by them. The terminal consumption included a "remainder" which varied from six to twelve per cent of the initial oxygen consumption. Since the recovery process with which they were concerned had presumably run its course independently of this "remainder," which they attribute to minor disturbances of metabolism brought into play by the exercise, they used the oxygen consumption at the end of the recovery period as base, rather than the pre-exercise consumption.

We observed an excess oxygen "remainder" large enough to be significant in eight of our nineteen experiments of series A and G. "The remainder" ranged from 9 per cent to 40 per cent of the initial oxygen consumption, averaging about 24 per cent (see columns 3, 4 and 5 of table 3). The question at once arises whether the more marked decline in oxygen consumption in the experiments which showed no "remainder" might have been due to a falling off in the condition of the experimental animals in the late stages of these experiments. So far as the arterial pressure is a valid criterion of metabolic state it is clear from the arterial pressure data given in table 1 that the absence of an oxygen "remainder" in some of our experiments cannot be thus explained. Thus, there was

no significant "remainder" in experiments A 10, A 17, G 2 and G 4, although the arterial pressure was high throughout them. On the other hand, "remainders" were seen in experiments A 16 and G 8, in which the arterial pressure was definitely lower.

To obtain the figures in column 7, table 3, we considered the smallest excess oxygen consumption observed late in the recovery period to represent the "remainder" and calculated the "recovery" oxygen consumption

TABLE 3

Excess oxygen consumption compared with disappearance of blood lactates

A, amytal; G, brain-stem transection

EXPERIMENT	BODY WEIGHT	BASAL O_2 CONSUMPTION PER MINUTE	OXYGEN REMAINDER		TOTAL EXCESS O_2 CONSUMPTION		LACTATE DISAPPEARING FROM BLOOD	
			Cc. per minute	Per cent of basal O_2	As observed	In excess of the "remainder"	Per 100 cc.	Total
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
		cc.			*cc.*	*cc.*	*mgm.*	*mgm.*
A 8	8.1	39	8	20	765	75	4.9	20
A 16	10.5	61	9	15	825	75	10.7	53
G 3	20.0	170	15	9	2,570	1,020	7.4	74
G 6	11.8	86			510	510	12.9	76
A 11	8.5	55			765	765	18.6	80
G 2	13.0	127			165	165	13.4	87
A 14	13.6	77			270	270	14.4	98
G 1	12.0	88			30	30	21.6	129
A 17	12.8	75			240	240	21.1	135
G 8	8.9	50	20	40	1,875	75	32.0	142
A 6	11.7	55	16	29	1,605	165	27.2	159
G 10	9.0	69			525	525	36.5	164
G 7	16.0	76			690	690	20.9	167
A 9	16.4	71	26	37	3,330	990	23.1	189
A 12	15.5	71	23	32	2,295	195	24.9	193
G 9	8.7	60			585	585	44.3	193
A 10	16.6	89			1,275	1,275	26.8	207
G 4	16.4	116			1,420	1,420	33.8	277
A 15	13.6	58	7	12	925	255	44.4	300

from that figure as base. Late increases in oxygen consumption, such as were seen in several experiments, were disregarded.

Column 9, table 3, gives the calculated decreases in blood lactates in the different experiments. These were determined on the assumption that the blood constitutes 5 per cent of the body weight in dogs as in man (Lindhard, 1926). The decreases from the maximum in milligrams per 100 grams blood (column 8, table 3) are readily obtained from the data of table 2. The total decrease in blood lactates is obtained by

multiplying these by the calculated blood weights. A. V. Hill and associates have assumed that the blood lactates bear a simple ratio to the total body lactates (Hill, Long and Lupton, 1924b). For the purposes of our problem it is not necessary, therefore, that an attempt be made to compute the actual decrease in body lactates.

To facilitate comparison of the oxygen and lactate data the experiments are arranged in table 3 in increasing order of lactate disappearance. Simple inspection of the table shows that neither the observed excess oxygen consumption (column 6), nor the excess consumption after allowance is made for the "remainder" in the experiments in which one occurred (column 7), bears such a direct relationship to the amount of lactate disappearing (column 9), as one would expect to find were the two as immediately interdependent as the theory of A. V. Hill states.

Furthermore, in the experiments in which a "remainder" was noted, the excess oxygen consumption attributable to it greatly overbalanced the consumption which would be attributed to lactate elimination. In these experiments some other influence than that exerted by lactates would be considered to account for the major part of the excess oxygen consumption following exercise. To maintain that in the experiments in which no remainder occurred the entire excess oxygen consumption was determined by lactate elimination seems to us unwarranted, particularly in view of the absence of anything more than the roughest of correlations between the amounts of oxygen consumed and the amounts of lactate disappearing.

We accordingly infer that in dogs under the experimental conditions here described the excess oxygen consumption following a bout of exercise is not determined, either in whole or in demonstrable part, by the amount of lactate set free during the exercise and got rid of after it is over.

SUMMARY

1. Changes in oxygen consumption and concentration of blood lactates consequent on exercise were studied in anesthetized dogs. Arterial pressures and rectal temperatures were recorded throughout all experiments.

2. Oxygen consumption increased greatly during exercise and continued high, with gradual decline, after it was over. Blood lactates also increased during exercise and fell off gradually thereafter.

3. After allowing for increase due to heightened body temperature an excess oxygen consumption, attributable to the exercise *per se*, was noted in nearly all experiments.

4. In nearly half of the experiments some excess consumption continued till the end, suggesting in this respect the "remainder" noted by Hill, Long and Lupton.

5. Neither the total excess oxygen consumption nor the excess after allowance was made for the "remainder" showed a quantitative relationship to the quantity of lactate disappearing from the body as deduced from the observed decline in blood lactates.

6. The inference is drawn that under the conditions here employed the excess oxygen consumption following exercise is not determined by the amount of lactate disappearing from the body concurrently.

7. About half of the dogs used in these experiments were anesthetized with amytal; the others by brain-stem transection under transient ether. No significant differences in results were seen in the one group as compared with the other.

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VARIOUS HEMOGLOBINS AND THEIR RENAL THRESHOLDS IN THE DOG

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In a study of hemoglobin conservation previously reported (1927) Whipple and Robbins observed a great difference between blood hemoglobin and muscle hemoglobin when given intravenously in anemic dogs. These dogs would tolerate more than ten times the dose of blood hemoglobin as compared with muscle hemoglobin before hemoglobinuria was observed. It was obvious that the renal thresholds for these two hemoglobins of the dog were quite different and this was accepted as an important physiological difference between substances which as tested chemically are so very similar or even indistinguishable.

This paper gives a sufficient number of experiments to establish the renal thresholds for dog blood hemoglobin and dog muscle hemoglobin as well as sheep and goose blood hemoglobin. It is obvious from a glance at chart A that the renal threshold for dog blood hemoglobin is highest and for dog muscle hemoglobin is very low while the thresholds for sheep and goose hemoglobins fall about midway between these extremes. As might be expected from these threshold values we observe a very brief disappearance curve for muscle hemoglobin—chart B.

There is an extensive literature pertaining to the production of experimental hemoglobinuria by the injection of hemoglobin. This literature up to 1916 is adequately reviewed by Sellards and Minot (1916). The methods of various workers differ so widely that no accurate comparison can be made. There is, however, fairly general agreement that experimental animals may be injected with hemoglobin in amounts sufficiently great to induce hemoglobinuria with safety provided that the injection mass be stroma free.

The amount of laked dog blood, intravenously injected which would cause hemoglobinuria in dogs was found by Ponfick (1875) to be 1.2 to 1.3 gram per kilo of body weight. He believed the renal threshold for laked sheep blood to be the same.

Sellards and Minot (1916) found that the hemoglobin obtained from about 25 cc. of packed human red blood cells could be injected into normal

humans without symptoms. The injection of amounts greater than this caused hemoglobinuria. These workers also found that this renal threshold was much lower in individuals whose serum contained the free hemoglobin resulting from rapid blood destruction.

Pearce, Austin and Eisenbrey (1912) in connection with some work on splenectomy calculated that a hemoglobinemia of 60 mgm. per kilo of body weight was sufficient to cause hemoglobinuria in dogs—compare chart A. This calculation assumed a certain rapid and constant rate of disappearance of hemoglobin from the blood stream that the direct observation of subsequent workers does not confirm.

Few animal experiments have been done using hemoglobin intravenously derived from blood foreign to that species. These few observations are cited by Von Starck (1898) in his discussion of the therapeutic value of the injection of man with crystallized horse hemoglobin.

Camus and Pagniez (1902) note that much less dog muscle hemoglobin will cause hemoglobinuria in dogs than is required with dog blood hemoglobin. These authors also noted that the amount of muscle hemoglobin necessary to cause a macroscopic hemoglobinemia is far above the renal threshold. This same phenomenon was noted by Achard and Feuille (1911) and thought by them to mean that the hemoglobinuria was of toxic origin and not related to the hemoglobinemia. This view has not met with general acceptance. We have been unable to find any comparative study of the renal thresholds of experimental animals following the injection of foreign hemoglobins.

Methods. Normal mongrel dogs were used in all but one experiment. In this exception (dog A) the animal had been previously made anemic by repeated bleeding.

Hemoglobin was obtained from four sources: normal dog blood, normal sheep blood, normal goose blood and normal dog muscle. The choice of sheep and goose hemoglobins was purely one of convenience. The sheep represents an unrelated animal easily bled. The goose represents a species in which the red cells are nucleated and hence presumably widely divergent from mammalian forms. A goose may be bled with relative ease from one of the large veins beneath the wing.

In all cases the blood was collected in an isotonic oxalate solution, one to nine parts of blood, and then washed twice in normal saline, using a high speed centrifuge. For laking, two volumes of distilled water were added to one volume of washed packed red blood cells. The stroma was separated by high speed centrifugalization. In goose blood this stroma is very abundant and separation is more thorough if sufficient solid sodium chloride to make the solution isotonic is added after hemolysis prior to the final centrifugalization. Separation of stroma may also be facilitated by saturating the hemoglobin solution with carbon dioxide followed by

centrifugalization. Both methods were used. As a routine all hemoglobin solutions to be injected were made isotonic either by the addition of a solid sodium chloride or its 10 per cent water solution, although no differences were observed when this procedure was not followed. Smith (1920) has shown that relatively large amounts of hypotonic fluid may be injected into dogs without causing detectable hemolysis.

The pigment content of the solutions injected was determined by the acid hematin method of Robscheit-Robbins (1920). Aliquot samples were taken, acidified, set in the ice box over night and compared with a known standard the following day. This standard is prepared from a hemoglobin solution whose pigment content has been determined by the method of Van Slyke. The muscle hemoglobin was prepared in the manner described by Whipple (1926).

To avoid possible foreign protein reactions, dogs were first desensitized by the administration of increasing doses of foreign hemoglobin during a three weeks period. Two doses a week are given. The first two, 0.05 gram and 0.1 gram respectively, were given intramuscularly. The second week the same amounts diluted to 10 cc. were given in the same sequence intravenously. During the third week two doses of 0.25 and 0.5 gram respectively were given intravenously.

Toxic reactions were occasionally seen. Early in the fourth week following the first desensitizing dose of sheep hemoglobin two animals gave symptoms referable to the protein being injected. Each dog sneezed repeatedly and then had mild convulsions lasting for several minutes. One animal had two such attacks on successive days. All of the dogs showed some symptoms of intoxication occasionally. These reactions occurred irrespective of the hemoglobin used and not related to the time lapse between its preparation and use. It not infrequently happened that out of four dogs receiving the same hemoglobin, one alone might show toxic symptoms. Barratt and Yorke (1914) have shown that the toxicity of hemoglobin solutions is related to stroma content but there is also considerable variation in the reaction of various dogs. One dog, E, suffered in this respect more frequently than any of the others. This dog had at that time a moderately severe follicular mange.

In all cases the hemoglobin was injected into a jugular vein. The injections were routinely made by burette and gravity, though for injection masses of a few cubic centimeters syringes were used and for volumes of 100 cc. or more a perfusion bottle was used.

There is considerable evidence to show (Barratt and Yorke, 1914) that injected hemoglobin may cause cast formation, and mechanical damage to the kidneys causing a temporary anuria. To minimize this danger each dog was given from 200 to 500 cc. of water prior to the hemoglobin injection. This dose was repeated at the end of the second or third hour

provided that no urine had been passed. Due to the possibility of trauma of the urinary tract with a resultant hematuria, catheters were not used.

After injection the dogs were placed in metabolism cages and closely watched and the first urine passed was promptly examined for hemoglobin. The guaiac reaction, first used as the criterion of a hemoglobinuria, was soon abandoned. It was found that most male dogs had a sufficient balanitis to give the urine a positive guaiac reaction. The technic finally adopted may be described as follows: The urine is immediately filtered and poured into the cups of a standard Dubosecq colorimeter. The eye-piece of the colorimeter is replaced by a Leitz micro-spectrometer. The appearance of the characteristic absorption bands of oxyhemoglobin indicates a hemoglobinuria. When practical the actual amount of hemoglobin excreted is calculated by comparing the urine with a known solution of hemoglobin. The colors are read in a standard colorimeter through a green filter as described by Kennedy (1926). It is important that the urine be examined soon after excretion as a green precipitate soon forms obscuring the characteristic spectrum. Following hemoglobin injection urine specimens were occasionally examined microscopically for blood cells and prior to the tabulated experiments control specimens were frequently checked by the spectrometer. No fixed routine was followed as to the sequence of amounts or varieties of hemoglobin injected. In all cases the interval between injections was at least twenty-four hours.

In comparing the rates at which various hemoglobins leave the blood stream, the following procedure was followed: Two samples of blood are removed through the injection needle by well oiled syringes. The first sample is discarded and the second immediately transferred to a centrifuge tube containing isotonic sodium oxalate solution. This transfer must be done with care to avoid hemolysis. After centrifugalization for half an hour at 2200 R.P.M. the resulting plasma should be water clear. The injection needle is left in place for four minutes after the injection and a third sample taken with the same precautions as before. Subsequent samples are removed hourly, always using two syringes and discarding the first sample. After separation the plasmas are pipetted into clean test tubes and the hemoglobin content calculated in a colorimeter. The first sample after injection is used as standard and is diluted as necessary. This in turn is compared with the injected hemoglobin solution, suitably diluted. Here as with the urine, readings are made through a green filter because of the presence of yellow bile pigment in later plasma samples.

EXPERIMENTAL OBSERVATIONS. In determining the renal threshold for hemoglobin we did not attempt to determine the most minute trace of hemoglobin which could be detected in the urine but in all cases gave sufficient hemoglobin to cause a distinct hemoglobinuria recognized readily by the eye and giving distinct hemoglobin bands in the spectrophotometer.

This amount of hemoglobinuria is indicated by (+) in all tables. Larger amounts of hemoglobin in the urine are indicated arbitrarily by two or more plus signs. In many of these experiments the amount of hemoglobin excreted in the urine was measured quantitatively but not recorded in the tables as not essential to this particular discussion. In many of the experiments the hemoglobin threshold is determined between very narrow

TABLE 1

Renal hemoglobin thresholds

Dog A, Normal. Weight 18 kilos

TOTAL VOLUME INJECTED	TOTAL HEMOGLOBIN INTRAVENOUS	HEMOGLOBIN PER KILO BODY WEIGHT	RENAL THRESHOLD PER KILO WEIGHT	URINE HEMOGLOBIN SPECTRUM
		Dog hemoglobin		
cc.	*grams*	*mgm.*	*mgm.*	
500.0	1.00	57.0		0
14.0	1.58	90.0		0
17.0	2.92	166.0		0
24.0	3.50	200.0		++
30.0	4.20	240.0	190.0	++++
		Sheep hemoglobin		
500.0	1.00	57.0		0
13.0	1.20	68.5		0
12.5	1.80	103.0		0
11.1	2.20	126.0		++
17.5	2.50	143.0	115.0	++++
		Goose hemoglobin		
500.0	1.00	57.0		0
30.0	1.52	88.0		0
17.5	1.75	100.0		0
31.5	2.00	115.0		++
48.0	2.66	152.0	105.0	++++
		Muscle hemoglobin		
100.0	0.15	8.6		0
157.0	0.22	12.8		++
160.0	0.27	15.6		+++
600.0	0.74	42.2	11.0	++++

limits while again time did not permit of a large enough series of injections to determine the blood hemoglobin threshold to within 5 mgm. per kilo. In no instance does this possible error exceed 5 per cent and the reactions are remarkably uniform for each individual dog. There may be considerable individual variations which we cannot explain but work is in progress to ascertain what factors may modify this threshold value of the normal dog.

Much to our surprise severe *anemia* did not modify these threshold values for blood hemoglobins—table 2. This is significant because the anemic dog is competent to utilize injected hemoglobin to build new red cells in its own system. In fact the anemic dog can conserve almost all the injected dog blood hemoglobin and recast it into new red cells (Whipple and Robscheit-Robbins, 1927). Moreover, the anemic dog can conserve for new red cell construction *foreign blood hemoglobin* when given intravenously. These experiments will be published in the near future. In view of this *utilization* of injected hemoglobin in anemia as contrasted with

TABLE 2

Renal hemoglobin thresholds

Dog A, Severe anemia. Weight 18.0 kilos

TOTAL VOLUME INJECTED	TOTAL HEMOGLOBIN INTRAVENOUS	HEMOGLOBIN PER KILO BODY WEIGHT	RENAL THRESHOLD PER KILO WEIGHT	URINE HEMOGLOBIN SPECTRUM
Dog hemoglobin				
cc.	*grams*	*mgm.*	*mgm.*	
27.0	3.00	172.0		0
31.0	3.25	186.0		0
73.3	3.50	200.0		+++
42.2	4.00	243.0	190	++++
Sheep hemoglobin				
15.8	1.90	107.0		0
19.0	2.00	115.0		+
15.0	2.20	125.0		++
20.6	2.70	155.0	110	++++
Goose hemoglobin				
500.0	1.00	57.0		0
30.2	1.75	100.0		0
31.7	1.90	109.0		++
38.2	2.00	115.0	105	++++

disposal of injected hemoglobin as bile pigment by the non-anemic dog, we anticipated an elevation of the renal threshold as a reasonable probability. Obviously the interesting conservation of hemoglobin in anemia does not depend upon any change of renal threshold but upon modification of the internal pigment metabolism about which our information is distressingly meagre.

Table 1 gives the renal threshold values of a normal dog using blood hemoglobin of the dog, sheep and goose and dog muscle hemoglobin. The muscle hemoglobin threshold is not established as accurately as in other animals but it is obviously lower than the other dogs. Likewise the

dog blood hemoglobin threshold is lowest in this dog but the difference is not nearly as great. We can give no reason for these individual variations.

Table 1 indicates that the injection of hemoglobin in large or small volumes of saline does not alter the renal threshold for hemoglobin. The introduction of 500 cc. saline intravenously with or without hemoglobin will cause a marked diuresis but this does not change the hemoglobin threshold, at least within the limits of these experiments.

Table 2 shows the *same renal threshold values* for blood hemoglobin in severe *anemia.* The same dog A was used as tested in table 1. The

TABLE 3

Renal hemoglobin thresholds

Dog D, Normal. Weight 22.2 kilos

TOTAL VOLUME INJECTED	TOTAL HEMOGLOBIN INTRAVENOUS	HEMOGLOBIN PER KILO BODY WEIGHT	RENAL THRESHOLD PER KILO WEIGHT	URINE HEMOGLOBIN SPECTRUM
		Dog hemoglobin		
cc.	*grams*	*mgm.*	*mgm.*	
28.9	4.00	169.0		0
49.0	4.50	190.0		0
42.2	4.75	201.0		++
50.0	5.00	212.0		++++
500.0	1.00	42.5	198.0	0
		Sheep hemoglobin		
22.0	2.20	94.0		0
19.2	2.30	97.5		0
18.7	2.40	102.0		++
21.0	2.50	106.0		+++
500.0	1.00	42.5	100.0	0
		Muscle hemoglobin		
125.0	0.11	4.6		0
200.0	0.34	14.5		0
244.0	0.35	14.8		+
250.0	0.39	16.5	14.6	++++

anemia was produced carefully over a period of 40 days; abstracting from day to day considerable amounts of blood by needle in the jugular vein. The blood hemoglobin level was kept between 30 and 50 per cent hemoglobin for a month prior to these hemoglobin injections given in table 2. The anemia level was 30 to 50 per cent hemoglobin as contrasted with 115 per cent hemoglobin, the normal blood hemoglobin for this animal. The dog was in all other respects normal, active and retained a good appetite. He survived the experiment without any complications.

We have accumulated considerable data related to sheep and goose blood

hemoglobin injections in severe anemia in dogs but there is no evidence of an elevated renal threshold for hemoglobin even in severe anemia lasting 2 to 3 years. It is of course possible that an anemia might be sufficiently severe to cause epithelial degeneration in the kidney which might modify this renal hemoglobin threshold but this degree of anemia and malnutrition is not reached in our experiments.

TABLE 4

Renal hemoglobin threshold

Dog E, Normal. Weight 13.1 kilos

TOTAL VOLUME INJECTED	TOTAL HEMOGLOBIN INTRAVENOUS	HEMOGLOBIN PER KILO BODY WEIGHT	RENAL THRESHOLD PER KILO WEIGHT	URINE HEMOGLOBIN SPECTRUM
Dog hemoglobin				
cc.	*grams*	*mgm.*	*mgm.*	
70.0	2.29	175.0		0
22.3	3.10	236.0		0
51.6	3.10	236.0		+
66.0	3.30	256.0	236.0	++++
Sheep hemoglobin				
500.0	1.00	76.0		0
18.5	1.80	137.0		0
13.3	1.90	145.0		+
20.0	2.00	152.0	140.0	+++
Goose hemoglobin				
	1.80	137.0		0
31.7	1.90	145.0		0
44.0	2.00	158.0		++
39.5	2.12	162.0	150.0	+++
Muscle hemoglobin				
100.0	0.15	11.5		0
100.0	0.16	11.9		0
120.0	0.20	15.6		+
178.0	0.22	17.2	15.0	+++

Table 3 shows other experiments with another normal dog, D. The threshold values of this dog are all determined within very narrow limits. The threshold value for muscle hemoglobin is higher than in dog A in table 1 but the other values for dog and sheep blood hemoglobin are in close accord with table 1.

Table 4 shows some interesting differences when compared with tables 1 and 3. The dog shows a distinctly higher renal threshold for blood hemoglobin, whether dog, sheep or goose, when compared with dogs A

and D. These thresholds are determined within narrow limits. As pointed out elsewhere these are individual variations which we cannot at present explain.

Table 5 shows experiments with 2 normal dogs. We note individual differences in the renal threshold for dog blood hemoglobin although the renal threshold for each dog is determined within the usual limits. We

TABLE 5

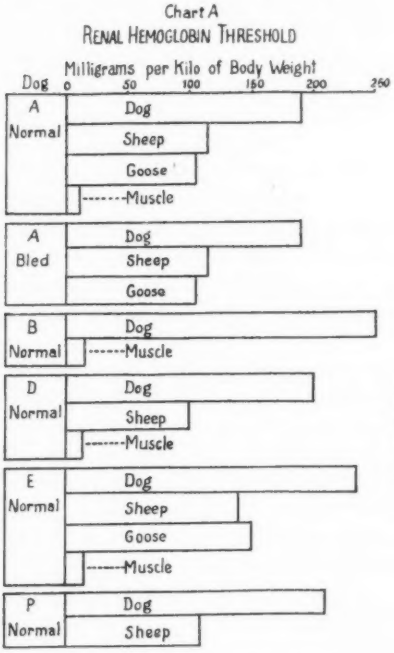
Renal hemoglobin thresholds

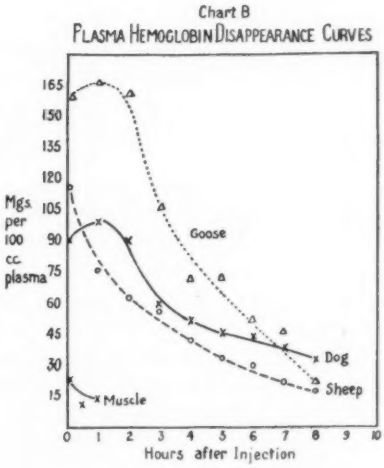
TOTAL VOLUME INJECTED	TOTAL HEMOGLOBIN INTRAVENOUS	HEMOGLOBIN PER KILO BODY WEIGHT	RENAL THRESHOLD PER KILO WEIGHT	URINE HEMOGLOBIN SPECTRUM
Dog B, Normal. Weight 13.0 kilos				
Dog hemoglobin				
cc.	*grams*	*mgm.*	*mgm.*	
12.0	1.50	115.0		0
25.0	3.15	240.0		0
28.0	3.40	260.0		++
24.0	3.50	268.0	250.0	++++
Muscle hemoglobin				
100.0	0.15	11.5		0
134.0	0.20	15.2		+
120.0	0.20	15.6		0
150.0	0.23	17.8	15.2	+++
Dog P, Normal. Weight 4.5 kilos				
Dog hemoglobin				
13.8	0.90	200.0		0
21.0	1.00	222.0		++
11.6	1.10	245.0		++++
12.0	1.20	267.0	210.0	++++
Sheep hemoglobin				
3.2	0.45	100.0		0
4.0	0.50	111.0		0
3.4	0.50	111.0		++
5.0	0.65	144.0	110.0	++++

note in dog B that almost equivalent amounts of muscle hemoglobin gave a negative urine one day and a positive at another time. When the threshold value is reached we may observe this phenomenon, evidently the balance being a very delicate one. Similar findings are observed in dog P—table 5, where the renal threshold for sheep hemoglobin is 110 mgm. per kilo. On one occasion this dosage gives a hemoglobinuria and again negative urine. What factors determine the escape of hemoglobin in the

urine under these conditions of delicate balance we are unable to say at present. At any rate shifting the hemoglobin dosage a few milligrams up or down will give a uniformly positive or negative urine as shown in the other tables.

Chart A shows graphically the absolute threshold values for the different hemoglobins and it is at once apparent that these different hemoglobins are uniform in their behavior within the blood stream. Dog A whether normal or very anemic presents the same threshold values. That these threshold values for the passage of hemoglobin through the kidneys are so





constant may be surprising but compare favorably with the sugar renal threshold. This may be recognized as another indication that the conservation of blood hemoglobin is an important business because this threshold in the normal dog is far above the low levels of circulating hemoglobin due to wear and tear or physiological wastage of red cells and hemoglobin.

Chart B shows the characteristic disappearance curves of the various hemoglobins introduced into normal dogs. The total volumes injected were the same and all conditions were kept uniform. Many curves of this nature have been charted for different dogs. The disappearance curves for goose and sheep hemoglobin are a little steeper than for dog blood

hemoglobin. Muscle hemoglobin is in a class by itself and escapes with great promptness from the blood plasma due to the low renal threshold. These low values are difficult to read accurately.

The method described for studying the rate at which various hemoglobins leave the blood stream is not free from objections. Hemolysis, due to handling of the fragile red cells of the dog cannot be ruled out. However, chart B gives readings selected from several similar experiments to show that dog, sheep and goose blood hemoglobins leave the blood stream at approximately equal rates. Following the intravenous injection of 1 gram hemoglobin in 500 cc. saline there was a small but measurable amount present in the blood stream at the end of 8 hours. This is in striking contrast to the experiment with muscle hemoglobin in which the plasma was scarcely tinged at the end of the first hour following the injection of an approximately similar amount. This is in accord with the observations of Camus and Pagniez (1902).

Discussion. The renal threshold values for dog blood hemoglobin show a maximum of 250—a minimum of 190 and mean value of 215 mgm. per kilo body weight. Granting that the normal dog blood contains about 18 grams of hemoglobin per 100 cc. the mean threshold value of 215 mgm. represents approximately 1.2 gram of whole laked blood per kilo of body weight which is in harmony with the early estimate of Ponfick, 1.2 to 1.3 per kilo (1875).

The renal threshold values for sheep hemoglobin show a maximum of 140—a minimum of 100 and a mean value of 116 mgm. per kilo body weight.

The renal threshold for goose hemoglobin in only two normal dogs shows values of 105 and 150 mgm. per kilo body weight. This means that the renal threshold for goose and sheep hemoglobin is very similar and approximately 50 per cent of the renal threshold values for dog blood hemoglobin.

The renal threshold values for muscle hemoglobin show a maximum of 15—a minimum of 11 and a mean value of 13 mgm. per kilo body weight. These figures give a threshold value for muscle hemoglobin which is about 6 per cent of the value for dog blood hemoglobin.

It is recognized as difficult or impossible to differentiate between various hemoglobins by means of chemical methods. Kennedy and Whipple (1926) showed how similar are the spectrophotometric analyses of blood and muscle hemoglobin. There are very slight differences which alone have little significance but taken together with the evidence of the experiments in this paper indicate some difference in chemical structure of muscle and blood hemoglobins. These biological reactions are at present more delicate than the best chemical analytical methods. Furthermore the muscle hemoglobin of the dog has definite precipitin reactions which differentiate it from blood hemoglobin (Hektoen, Robscheit-Robbins and Whipple, 1928). On the other hand we know (Whipple and Robscheit-

Robbins, 1926) that muscle hemoglobin injected intraperitoneally, intramuscularly or intravenously will be in part promptly broken down to form bile pigment. This indicates obviously identity of certain constituent fragments in these various hemoglobins.

SUMMARY

When hemoglobin solutions are given intravenously to normal dogs certain amounts are tolerated before hemoglobinuria is observed. The point at which hemoglobin appears in the urine is called the *renal threshold* for the specific hemoglobin and the amount of hemoglobin in milligrams per kilo body weight readily ascertained. Under these experimental conditions this is a remarkably constant figure for any given normal dog.

The renal threshold value for dog blood hemoglobin is about 215 mgm. per kilo body weight—maximum and minimum values 250 and 190 mgm.

The renal threshold for sheep and goose hemoglobin is about 115 mgm. per kilo body weight or approximately one-half the dog hemoglobin threshold values.

The renal threshold value for dog muscle hemoglobin is very low—11 to 15 mgm. per kilo body weight. This amount is but 6 per cent of the value given for the renal threshold of dog blood hemoglobin.

Severe, prolonged anemia does not modify these renal threshold values for these various blood hemoglobins.

These experiments give added evidence that muscle hemoglobin is quite different biologically from blood hemoglobin. We may believe that muscle hemoglobin is foreign to the blood stream but evidence for this is inconclusive.

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THE DETERMINATION OF THE CARDIAC OUTPUT OF MAN BY THE USE OF ACETYLENE

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The various methods described in recent years for determining the cardiac output of man consist essentially either 1, in an application of the Fick principle, or 2, in measuring the rate with which either a normally present gas (nitrogen) or a foreign gas (nitrous oxide, ethyl iodide or ethylene) is exchanged during the passage of the blood through the lungs. Results obtained by the carbon dioxide methods, which are based on the Fick principle, have been criticized in previous publications (Marshall and Grollman, 1928; Grollman, 1928b), and the difficulty of measuring the rate of elimination of nitrogen has also been demonstrated (Marshall, Harrop, and Grollman, 1928). The idea entertained by Henderson and Haggard (1925) that in ethyl iodide they had found a gas which was completely destroyed in the organism before the completion of a circuit by the blood, has been shown to be erroneous. It is only by abandoning this idea and actually determining the amount of this gas in the venous blood, as has been done recently by Starr and Gamble (1928), that any values worthy of consideration have been obtained. In the case of the other foreign gases—nitrous oxide and ethylene—as used in the procedures of Krogh and Lindhard (Krogh and Lindhard, 1912; Lindhard, 1923, 1925) or of Marshall and Grollman (1928), the hope of any destruction of these gases was never entertained, and the methods were elaborated so as to complete the determination in the time of a single circuit of the blood. In the present communication the use of another gas, acetylene, is advocated for the determination of the cardiac output of man. This gas will be shown to have many advantages over the gases heretofore used both as regards ease of manipulation and degree of accuracy obtainable.

Comparative value of different gases. In the comparative evaluation of any gas for the determination of the cardiac output, aside from the considerations regarding the method itself, the following are the chief factors which must be considered: 1, the solubility of the gas in blood; 2, the variation of this solubility in the blood of different individuals; 3, the ease of manipulation of the gas, and 4 the analytical determination of the gas.

Solubility of gases in blood. The absolute value of the solubility of the

foreign gas in blood is of prime importance in determining its value for measuring the cardiac output. Gases which are too slightly soluble are unsuitable since sufficient accuracy is not attainable for detecting their rate of absorption. Hydrogen gas is an example of this type. The *desideratum* is a gas having a sufficiently great solubility so that with the amount absorbed no appreciable error will be introduced by the analytical method employed. Substances, such as ether, which are very soluble in blood are equally undesirable. Acetylene is intermediate as regards its solubility, between ethylene and nitrous oxide, on the one hand, and ethyl iodide on the other. The solubility of acetylene in blood at body temperature is 0.740 (expressed in terms of cubic centimeters of gas at 0° and 760 mm. dissolved in 1 cc. of blood) compared to 0.123 for ethylene and 0.405 for nitrous oxide. It is thus superior to both ethylene and nitrous oxide in so far as this factor alone is concerned, since from a given concentration of the gas, six times as much acetylene will be absorbed as ethylene and over one and a half times as much as nitrous oxide. Assuming that all three of these gases could be determined with the same degree of accuracy, the analytical error introduced in determining the amount of gas absorbed will thus be much less in the case of acetylene than with either ethylene or nitrous oxide.

The variation of the solubility of a gas in different bloods. The solubility of any given gas in different bloods is not a constant but varies to a certain degree, depending upon the nature of the gas, not only in the blood of different animal species but even in different individuals of the same species. In the use of a foreign gas for the determination of the cardiac output, the solubility or partition of the gas between the alveolar air and blood of the subject whose output is being determined must be known. For a gas to be generally applicable, therefore, its solubility should be constant within small limits of variation. The importance of this problem in connection with the measurement of the cardiac output may be briefly discussed.

With the exception of carbon monoxide and oxygen where actual combination occurs between the gas and hemoglobin, or of carbon dioxide, where bicarbonate is formed, most gases hitherto studied dissolve in blood according to the laws of solution of gases in liquids.[1] In considering the solubility of a given gas in so heterogeneous a system as blood, we must take into account the various elements composing the fluid, viz., the dis-

[1] Against the view presented here may be cited the work of Findlay and Creighton (1911) whose experiments on the solubilities of carbon dioxide, oxygen, carbon monoxide, nitrous oxide, and nitrogen in blood and serum, indicate adsorption of these gases by the blood colloids. Conant and Scott (1926) also found that the solubility of nitrogen in hemoglobin solutions followed Freundlich's adsorption formula.

solved salts, proteins, hemoglobin, and the lipoid constituents of the erythrocyte. So far as the salts, proteins, and hemoglobin are concerned, they seem to behave simply like dissolved substances which in general tend to decrease the solubility of the gas in question in the solvent. The lipoid of the erythrocyte, however, acts as a solvent of the gas and thus tends to increase the solubility of the gas in blood, as compared to an aqueous solution, because of its own solvent power. Although the concentration of lipoid in blood is only about 0.2 to 0.3 per cent, the preponderatingly greater solubility of certain (particularly the organic) gases in lipoids over their solubility in water makes the effect of the presence of this lipoid considerable. In general, we may predict the solubility of a gas in blood from its solubility in lipoid. Thus the solubility of hydrogen, carbon dioxide, nitrogen or oxygen, which are not very soluble in lipoids, will not be greatly different in blood than in water. The slight effect of the solvent power of the lipoid, in the case of these gases, is overbalanced by the decrease in solubility, as compared to water, which the presence of the dissolved salts, proteins, and hemoglobin produces. The sum total of these effects is a decreased solubility of these gases in blood as compared to water (Bohr, 1905: Van Slyke, Sendroy, Hastings, and Neill, 1928: Van Slyke and Sendroy, 1928).

Gases, such as ethyl iodide, chloroform, ether, etc., on the other hand, because of their *lipophilic* nature are much more soluble in blood than in water due to the solvent action of the lipoidal envelope of the blood corpuscles. In the case of ethylene, nitrous oxide or acetylene, we find a behavior, as regards their solubility in blood, which is intermediate between the relatively lipoid-insoluble oxygen, carbon dioxide, nitrogen, and hydrogen, and the highly *lipophilic* substances, ether, chloroform or ethyl iodide.

Ethylene is one and a half times as soluble in blood as in water, thus retaining the *lipophilic* character to a considerable degree. Nitrous oxide is much less *lipophilic* and its solubility in blood is only slightly higher than in water (Siebeck, 1909). In the case of acetylene we have the least *lipophilic* of all three of these gases, and its solubility in human blood is slightly less than its solubility in water at the same temperature.

If one considers now the effect of variation of the lipoid content of different bloods on the solubility of any given gas, it will be obvious that the more *lipophilic* the gas, the more will it be affected by any given change in the lipoid content of the blood. Thus the difference in solubility of ethyl iodide in human bloods of different individuals or of different animal species will be pronounced (Starr and Gamble, 1928). In the case of ethylene, likewise, this difference in solubility, though in no manner as pronounced as in the case of ethyl iodide, is nevertheless of practical importance and capable of introducing serious error in determinations of

the cardiac output (Marshall and Grollman, 1928). The solubility of nitrous oxide will not vary appreciably under these conditions. Acetylene, in this respect, has marked advantages over any of the foreign gases hitherto recommended for the determination of the cardiac output. *Normal variations of its solubility are negligible, being extremely small.*

In order to demonstrate the above considerations, the solubilities of ethyl iodide, nitrous oxide, ethylene, and acetylene in blood of various sources and in water are collected in table 1.

The data of this table (determined at 37 to 38°C.) were collected, in part, from the sources indicated. The remaining data were determined during the course of an investigation (*unpublished*) of the solubility of various

TABLE 1

The solubilities of ethyl iodide, ethylene, nitrous oxide and acetylene in water and blood fluids

SOLVENT	ETHYL IODIDE	ETHYLENE	NITROUS OXIDE	ACETYLENE§
Water	2.7*	0.078		0.747
Normal human blood	5.6 to 6.5*	0.120 to 0.126†	0.405‡	0.740
Anemic human blood	5.0 to 5.4*			0 735¶
Normal dog's blood	9.5 to 11.8*	0.147 to 0.158†		0.759

* Starr and Gamble (1927, 1928).

† Marshall and Grollman (1928).

‡ Lindhard (1925).

§ The solubility of acetylene in a sample of blood from a patient with *polycythemia vera* (kindly furnished by Dr. Geo. A. Harrop) was found to be 0.710. The R.B.C. of this blood was 6,800,000.

¶ This blood was obtained from a patient with *myeloid leucemia* in whom there was an anemia (R.B.C., 2,200,000) and a relative lipaemia.

gases in blood and blood-fluids and were made at 37.50° ±0.05°. Reference to table 1 shows the obvious superiority of acetylene over any of the other gases used for determining the cardiac output, in so far as its solubility relationships are concerned.

Manipulation of gases. Ease of manipulation is an important factor in establishing the practical value of a gas for determining the cardiac output. Ethylene and nitrous oxide require no especial precautions and are easily manipulated under the usual laboratory conditions. Ethyl iodide, however, must be kept inaccessible to rubber or greases, which introduce considerable errors by absorption of this gas (Starr and Gamble, 1928). Acetylene, like ethylene and nitrous oxide, does not pass through rubber rapidly nor is it absorbed appreciably by such substances as stop-cock grease.

It must be pointed out that acetylene suffers the possible disadvantage that, under certain conditions, it is a violent explosive. With ordinary

precautions, however, and particularly since the method of determining the cardiac output does not involve the use of flames or burning the gas, no danger is attached to its use. A method is later described in which sufficient gas for determining cardiac outputs can be easily prepared in the laboratory so that the necessity of keeping the gas in tanks under pressure (in which condition alone, any violent explosion is probable) is obviated.

The ease of the analytical determination of acetylene as compared to other gases. For practical value in determining the cardiac output, a foreign gas must be easily and accurately analyzable. In the case of ethylene, this analysis is comparatively easy when high concentrations of the gas are used. However, the nauseating taste of such concentrations and the other factors already mentioned mitigate against its use. The analysis of ethyl iodide to be accurate involves rather complicated manipulations (Starr and Gamble, 1928).

The greatest objection to the use of nitrous oxide for determining the cardiac output and the factor which has mitigated against its general use, has been the difficulty of carrying out analyses of this gas. Although in the hands of one experienced in the technique of manipulating gases, the analysis of nitrous oxide can be carried out with a relatively high degree of accuracy, the analysis can not be said to be of extreme simplicity. Indeed, when one considers the precautions necessary for accurate work—the complete removal of all traces of oxygen before burning the nitrous oxide, the avoidance of overheating, etc.,—it is not surprising that because of the difficulty experienced in the analysis, objections have been raised against the method (Boothby and Sandiford, 1915). Moreover, the necessity of storing oxygen-free hydrogen for the combustion, the effect of organic substances such as grease from the stop-cock, etc., the necessity of having stop-cocks which do not leak under about 60 millimeters of mercury pressure—are all mechanical details which make the analysis of nitrous oxide difficult and to the physiological or clinical investigator, often impossible without long practice.

In the case of acetylene, fortunately, one has available an extremely simple method of analysis, which shall be described in a later section of this paper. The final accuracy obtainable is of the order obtained in the case of carbon dioxide or oxygen analysis.

We thus see that acetylene as compared to either ethylene, nitrous oxide, or ethyl iodide has marked advantages which should render it admirably suitable for determining the cardiac output. The results obtained by its use, as described below, show it worthy of this confidence in its superiority.

Procedure in determining the cardiac output. The procedure followed in the present work was that described previously (Marshall and Grollman, 1928). The only apparatus necessary for the determination by this

method consists of a rubber bag,[2] attached to a three-way tap such as is used in ordinary metabolism experiments. Into the rubber bag is introduced approximately half a liter of acetylene, obtained by one of the methods described below. The quantity of acetylene used need not be measured or even accurately estimated since its absorption is such that accurate results can be obtained with a final gas concentration, varying from about 4 to 20 per cent. About 8 to 10 per cent, however, is a very convenient concentration. Approximately one hundred cubic centimeters of oxyen are now introduced and the bag is almost filled with air, giving a total volume of gas mixture equal to about two and a half liters. Since none of the gas quantities need be exact the gas mixture is easily prepared, the amounts of the various gases being judged sufficiently accurately without the necessity of measurement.

After determining (by any of the usual methods) the oxygen consumption of the subject whose cardiac output is to be estimated, the above described gas is rebreathed for a period sufficiently long enough to bring about mixing in the *lung-bag* system, as described previously (Grollman and Marshall, 1928; Grollman, 1928b), and a sample is taken in an evacuated tube. After about 5 more seconds of rebreathng, a second sample is taken. An analysis of these two gas samples and the barometric pressure give the necessary data for calculating the arterio-venous oxygen difference. This calculation is made by means of the following equation:

$$\text{Arterio-venous oxygen difference} = \frac{(O_2)_{\text{Dif.}}}{(C_2H_2)_{\text{Dif.}}} \times 740 \times \frac{B-48.1}{760} \times \frac{(C_2H_2)_{\text{av.}}}{100}$$

or combining the numerical terms:

$$= 0.00974 \times \frac{(O_2)_{\text{Dif.}}}{(C_2H_2)_{\text{Dif.}}} \times (C_2H_2)_{\text{av.}} \times (B-48.1)$$

In the last equation the arterio-venous oxygen difference, in terms of cubic centimeters of oxygen per liter of blood, is expressed in terms of the corrected difference in the oxygen $[(O_2)_{\text{Dif.}}]$ and acetylene $[(C_2H_2)_{\text{Dif.}}]$ concentrations (expressed as per cent) in the two samples of gas taken during the experiment, the corrected barometric reading in millimeters of mercury (B) minus the vapor tension of the water vapor in the lungs

[2] This bag should have a capacity of approximately 2.8 liters. The elliptically shaped bag, used in anesthesia but having a width of 12 cm. and a length of 27 cm. is appropriate. One end of the bag should have a short piece of rubber tubing (about 5 mm. in diameter) through which the bag is filled. The other end should have a collar about 2½ cm. in diameter. This should be rather short (1 cm.) so as to avoid the collapse of the bag, with consequent interference in the mixing, during the preliminary rebreathing period.

(48.1 mm.)[3] and the factor 0.00974. The latter is derived by combining the constants 760 and 100, and 740, the solubility of acetylene (expressed in terms of cubic centimeters at 0° and 760 mm.) in blood at body temperature. This solubility value is the average determined on two individuals and is in close agreement with the value found by Schoen (1923).

The analysis of acetylene-air mixtures. For the analyses of the gas mixtures, the ordinary type of gas analysis apparatus used in metabolism work is utilized. To the usual carbon dioxide and oxygen absorbers is added a third absorber for acetylene.

Carbon dioxide is first determined by the usual method of absorption in alkali. To avoid loss of acetylene by solution in the carbon-dioxide absorbing fluid, the latter should consist of a 10 per cent alkali solution, saturated with sodium chloride or nitrate. By manipulating both samples in the same manner during the absorption of the carbon dioxide, the small errors introduced by the slight solubility of the acetylene in the absorber are counterbalanced.

After absorption of the carbon dioxide, the acetylene is determined. The absorption fluid for this gas is prepared (Treadwell and Tauber, 1919) by dissolving 20 grams of mercuric cyanide and 8 grams of sodium hydroxide in 100 cc. of water. This absorbent takes up acetylene with extreme rapidity and completeness forming a white precipitate of mercuric carbide.[4] The oxygen is finally determined by the usual method of absorption in pyrogallol. The ordinary gas pipette with a 7 cc. bulb and a stem calibrated in hundredths of a cubic centimeter over a range of 4 cc. permits the absorption of all three gases without danger of running beyond the calibration. When the calibration extends only over 3 cc., this may be avoided by storing a measured volume of nitrogen in the oxygen absorption pipette. In the present work, a pipette with a 20 cc. bulb and a stem calibrated in hundredths of a cubic centimeter over the range of 20 to 25 cc. was used. In this apparatus, 4 to 5 cc. of nitrogen were stored in the pyrogallol absorber before introducing the sample for analysis. The apparatus was also supplied with a Pettersson (1886) oil-bubble which

[3] The value 48.3 given in the paper of Marshall and the author (1928) referred to the vapor pressure of water at 37.5°. The present value, 48.1 refers to the vapor pressure of blood at this temperature (Grollman, 1928a). There is some confusion in usage as regards the temperature to be considered as occurring in the mixed venous blood coming to the lungs. This temperature would vary, possibly a degree or more, under extreme conditions, e.g., physical exercise or extreme changes in the external temperature (Bazett, 1927), and would necessitate changes in the solubility and vapor tension constants used in calculating the cardiac output. This effect on the vapor tension is negligible. Its effect on the solubility, in the case of acetylene, would amount to less than 2 per cent for a degree change in the temperature.

[4] This precipitate adheres tenaciously to the glass of the absorption pipette. It is easily removed, however, on recharging the pipette, by immersion for a minute in dilute acid and then rinsing in a stream of water.

permits of much more accurate readings than in the ordinary Haldane apparatus. With the above described apparatus, the analyses are in error less than 0.01 per cent. This refinement, however, is unnecessary for most work where, with the ordinary apparatus and an 11 cc. pipette, values which do not vitiate the final results more than 2 or 3 per cent should be obtainable.

The source of the acetylene. No difficulty was encountered in obtaining acetylene sufficiently purified to be safe for breathing. Such acetylene prepared for use as an anesthetic is procurable in Europe, but no American source of the gas, so purified, could be found. The acetylene used in this work was at first prepared by the interaction of water on commercial calcium carbide. The latter substance contains various impurities which liberate the physiological harmful hydrogen sulfide, ammonia, hydrogen phosphide, and hydrogen silcide. The repugnant odor of the gas derived from calcium carbide is due to the presence of these substances which must be removed to render the gas safe for breathing. A most satisfactory means of preparing pure acetylene, in small quantities, was found in the method described by Mathews (1900). The gas may be purified by passage through a solution prepared by dissolving 15.6 grams of crystalline copper sulfate ($CuSO_4 \cdot 5H_2O$) in 100 cubic centimeters of water and adding 5 cc. of 20 per cent sulfuric acid. This absorber is followed by another containing chromic acid in sulfuric acid solution, by one or more absorption towers filled with pumice stone saturated, as advocated by Wieland (1922), with a solution of cuprous chloride in hydrochloric acid and by a final tower, containing caustic soda, to remove any acid liberated. Acetylene prepared in the above way has been found by Wieland (1922) to be without toxic effect on the lower animals. It has been found perfectly satisfactory for use in determining the cardiac output. Although not possessing the "pleasant ethereal odor", which chemical treatises attribute to the pure gas, in no case has there been any difficulty in its use. This is in accord with the experiences of others who have used acetylene as an anesthetic (Brandt, 1926). The gas may be more simply obtained in a form suitable for breathing, by passing the ordinary commercial product through the train of absorbers described above. Acetylene, dissolved under pressure in acetone, is sold commercially and is readily obtainable on the market. This gas is used as an anesthetic after passage through a column of granular activated carbon (Goldman and Goldman, 1925), which removes the acetone and traces of the sulphur and phosphorus compounds present in the gas.[5]

[5] I am indebted to Dr. John S. Lundy, of the Mayo Clinic, who has used acetylene, prepared in this manner (*private communication*) for major surgical operations, for suggesting this source of the gas for physiological purposes. I am also indebted to Dr. A. B. Ray of the Union Carbide and Carbon Research Laboratories, for furnishing the details of the purification process by means of activated carbon.

Another method of preparing the gas in a very pure form is suggested by the observation of Kekulé (1864) who noted the formation of acetylene at the anode on the electrolysis of fumaric or maleic acids. The preparation of pure acetylene by the formation of copper acetylide from acetylene and ammoniacal cuprous chloride and the regeneration of the former has also been suggested (Meyer-Jacobson, 1907).

RESULTS. In the accompanying table (table 2) are given the results of duplicate determinations on six individuals. All the individuals studied

TABLE 2

Consecutive determinations of the cardiac output of normal individuals by the use of acetylene

SUBJECT	POSTURE	CONDITION	PULSE RATE PER MINUTE	OXYGEN CONSUMPTION	CONCENTRATION OF ACETYLENE IN THE FIRST SAMPLE	CIRCULATORY MINUTE VOLUME OF THE HEART
				cubic centimeters per minute	*volume per cent*	*liters*
J. G.	Recumbent	Basal	70	235	15.98	4.00
♂					8.46	3.93
A. G.	Recumbent	Basal	70	232	9.81	3.67
♂					4.48	3.72
A. L.	Recumbent	Basal	60	207	6.58	3.13
♀					5.15	3.20
L. C.	Sitting	Non-basal	62	208	4.49	3.31
♀					6.85	3.35
G. S.	Sitting	Non-basal	58	212	10.17	3.41
♂					9.46	3.46
E. G.	Sitting	Non-basal	68	218	7.40	4.66
♂					6.10	4.60

were young, healthy adults in the third decade of life, except G. S. who was 46 years old. Except for the subjects, A. L. and A. G., the other individuals studied had never performed the experiment previously. After one-half hour rest in the recumbent or sitting positions, as indicated, the first determination was made. After five minutes had elapsed the oxygen consumption was determined by means of the Krogh (1923) spirometer. Five minutes after the conclusion of this determination a second determination of the cardiac output was made. The last column in the table represents the cardiac outputs as calculated from a single analysis (no duplicate analyses were ever done) of the gas samples and

the oxygen consumption which was assumed to be constant throughout the course of the experiment (20 minutes).

The agreement of successive determinations is remarkable and shows a constancy of duplication never heretofore reported as obtainable by any method used for determining the cardiac output. There is every reason to believe that the *cardiac output, like other bodily functions, is constant for an individual in a relatively constant state of rest.* Wide variation of successive determinations on the same individual at the same sitting with no obvious nervous or excitatory reactions, should not occur and such variations when obtained can only indicate a variable error in the method. In the case of acetylene, as used in the present method, with an analytical error in the gas analyses of only 0.01 per cent, the final results should not be in error more than 1 or 2 per cent, and this is actually found to be the case in the results obtained. Of course, any error in the theory upon which the method is based may introduce a constant error so that the final results may possibly differ from the true cardiac output by a margin greater than that indicated in the variations of successive determinations from one another. The magnitude of the results obtained is of the same order as those obtained by the recent use of the Krogh-Lindhard method (Liljestrand and Stenström, 1925), by the method of Burwell and Robinson (1924), by Marshall and Grollman (1928), Grollman (1928b), and most recently, on two individuals by Starr and Gamble (1928).

The results differ markedly from the much higher values reported by Christiansen, Douglas, and Haldane (1914) and those who have used the method of Field, Bock, Gildea and Lathrop (1924). This wide divergence in the results obtained by the use of a foreign gas and by the various carbon dioxide methods is particularly striking and irreconcilable when one compares the results obtained in the recumbent posture (Grollman, 1928b).

Time allowable for the rebreathing procedure. The basis for the assumption that the procedure, as used in this investigation, gives correct values for the cardiac output of man, has been reported in detail in a previous communication (Marshall and Grollman, 1928) and need not be reconsidered here. The high degree of accuracy obtainable by the use of acetylene permits, however, a more satisfactory experimental test of the factor of the time allowable for the rebreathing procedure. Accurate knowledge of this point is of extreme importance to the method. Due to the large error involved in determining the cardiac output by means of nitrous oxide or ethylene when the collections are made over a very short period, it was considered worth while to repeat the experiments intended to settle this point by the use of acetylene, which permits accurate determinations when the samples are collected at intervals of even so short a period as five seconds. The results of this study are given in table 3.

After a preliminary period of rest, the determinations were made with an interval of ten minutes between each rebreathing.

The results of table 3 indicate that there is no drop in the cardiac output determined over the range of 15 (of 12, in the case of A. G. who could mix in this time) to 25 seconds after the beginning of the rebreathing. This substantiates the view of Marshall and Grollman (1928) as opposed to Lindhard (1925) who considers 15 seconds as the limit allowable for the experiment.

It may be mentioned here that the constancy of the cardiac output over a period extending to 25 seconds, does not necessarily indicate that no blood

TABLE 3

The circulatory minute volume as determined with different times of collection of samples of the acetylene-air mixtures

SUBJECT	TIME OF COLLECTIONS OF THE SAMPLES AFTER THE BEGINNING OF THE REBREATHING	CIRCULATORY MINUTE VOLUME INDICATED
	seconds	*liters*
A. L., ♀	15 and 20	2.80
	18 and 23	2.75
	20 and 25	2.79
	25 and 30	2.69
A. G., ♂	12 and 17	3.78
	17 and 22	3.80
	22 and 27	3.74
	27 and 32	3.52
G. S., ♂	15 and 21	3.87
	18 and 23	3.80
	23 and 30	3.49
J. G., ♂	15 and 20	4.04
	20 and 25	3.98
	25 and 30	3.74

has completed a circulation until this time. As a matter of fact, the two errors produced by a return to the lungs of venous blood containing acetylene will tend to compensate for one another. The return of acetylene will lower the value of the observed output. The decrease in arterio-venous oxygen difference (resulting from an increased blood flow due to the rebreathing procedure) will, on the other hand, tend to raise the observed values. Hence, by this compensation of errors, one might obtain (fortuitously, of course) the correct output, even after the completion of a circuit by the blood. This compensation is probably only effectual over a

period of very short duration and can have no serious importance on the interpretation of the observed results.

We may conclude from the results of table 3 that it is safe to prolong the experiment to 25 seconds after the beginning of the rebreathing. Actually, however, the prolongation to this time is not necessary since by the use of acetylene, five seconds (instead of 10 to 12, as in the case of nitrous oxide or ethylene) suffices as the time necessary to elapse between the taking of the two samples for analysis. Hence, by taking these samples at 15 and 20, or at 18 and 23 seconds after the beginning of the rebreathing, one may obtain accurate results even for the recumbent posture. Repetition of the experiment, with collections at 18 and 23, and at 15 and 20 seconds, respectively, will serve as a check for the beginner

TABLE 4

The cardiac output as obtained by the use of acetylene compared to the values obtained with nitrous oxide. The results are expressed in terms of liters per minute

SUBJECT	NITROUS OXIDE METHOD	ACETYLENE METHOD
A. G., ♂	3.6	3.65
	3.8	3.62
	3.5	3.69
A. C., ♀	2.8	2.76
	2.6	2.72
	2.9	2.68
E. G., ♂	4.2	4.45
L. C., ♀	3.2	3.02
J. G., ♂	4.0	4.30

using the method, to insure attainment of mixture and accuracy in technique.

Comparison of the results obtained by the use of acetylene and nitrous oxide. Since determinations of the cardiac output previously reported by Marshall and the author (1928) and the author (1928b) were made for the most part with nitrous oxide, it was considered of value to compare the results obtained by this gas with those obtained by the use of acetylene. The results of this comparison made on several individuals on whom single determinations were made by each method, are given in table 4. The several series of determinations on the subjects A. G. and A. L. were made on successive days. Considering the fact that the error in determinations by the nitrous oxide method is about 10 per cent while the error using acetylene is only about 2 per cent (as judged by the agreement of duplicate determinations), the agreement shown in table 4 is satisfactory. Satisfactory agreement between the results obtained by the use of low and high ethylene concen-

trations and nitrous oxide have been previously reported (Marshall and Grollman, 1928). The fact that with different methods, results of the same order of magnitude have been obtained by Liljestrand and Stenström (1925), by Burwell and Robinson (1924), by Marshall and Grollman (1928), by Grollman (1928b), and by Starr and Gamble (1928) indicates that we have available, at last, methods for obtaining essentially correct values for the cardiac output of man.

The use of acetylene in other methods. Acetylene is not limited in its use to the method followed in the present investigation. This gas would offer the same marked advantages over nitrous oxide, when used in the procedure of Krogh and Lindhard, as it does in the present method of Marshall and Grollman. It might also be suggested that acetylene might be used in place of ethyl iodide in the procedure of Starr and Gamble (1928). The great limitations to the use of the procedure of Marshall and the author are the long time necessary for mixing and the necessity of completing the determination in the time of a single circulation. These difficulties, of no importance in normal cases, render the application of the method to certain physiological and clinical problems impossible. The recent method of Starr and Gamble (1928) may find application to such cases. These authors report results of consecutive determinations on two subjects. The results obtained on one of these subjects agree within 0.1 liter, which would indicate an excellent degree of accuracy. The results on the second individual studied show, however, much wider variations. The possibility may be suggested, that with acetylene, which has neither the manipulative nor the analytical difficulties of ethyl iodide, the method as devised by Starr and Gamble may be of considerable practical value. The results of Schoen and Sliwka (1923) indicate, however, that acetylene (as might be expected from its general behavior in the body) accumulates very rapidly in the blood, complete saturation occurring (in the case of the rabbit) in seventeen minutes. This inability of the tissues to store acetylene would mitigate against the use of this gas by the method cited.

SUMMARY

Acetylene has been used for the determination of the cardiac output in man. The requirements of foreign gases as used in determining the cardiac output of man are discussed and it is shown that acetylene is much more ideal than ethylene, nitrous oxide, or ethyl iodide, the gases which have hitherto been used. This superiority of acetylene rests on its increased solubility over ethylene or nitrous oxide in blood, the constancy of this solubility as compared to these gases or ethyl iodide, and the ease with which analyses and other manipulations can be carried out with acetylene as compared to any of the other three gases. Acetylene possesses chemical,

physical, and physiological properties which render it distinctive in these respects. Consecutive determinations on the same individual showed a variation of about 2 per cent in the values of the cardiac output,—an agreement hitherto never obtained by any method applied to man. The effect of the time of rebreathing the acetylene-air mixtures, and the results obtained by the use of this gas as compared to nitrous oxide were also studied.

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THE INEFFECTIVENESS OF VAGINAL SMEARS IN PREDICTING THE OESTROUS CYCLE IN THE RABBIT[1]

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Stockard and Papanicolaou (1917) demonstrated that the guinea pig has a definite sexual period lasting about 24 hours and recurring every fifteen to sixteen days. The evidence for these facts was obtained by making histological examinations of the cellular content of the vaginal fluid and correlating the periodic changes which were observed, with tissue studies of the entire reproductive system, fixed at different stages, so that in the end, microscopic examination of the vaginal fluid gave an index to changes in any part of the generative tract. By this same vaginal smear method Long and Evans (1922) studied the oestrual cycle in the rat; Allen (1922) in the mouse; Hartman (1923) in the opossum; Corner (1923) in the monkey; Lehman (1921) in women, and King (1926) also in women.

Corner (1923) quotes Long and Evans as referring to unpublished work of Pelkin which gives evidence of a well-defined oestrual cycle in the rabbit, determined by the vaginal smear method. We are unable to find more than the above-mentioned reference to this work.

Since fixed tissue studies of the ovaries of rabbits with hypothyroidism give evidence of an increase in stroma, a diminished number of developing follicles and other signs of atrophy, it seems of interest to determine whether different degrees of thyroid activity might not predispose to a disturbance in the periodic function of the ovary, which by analogy from studies of other rodents, should be detected by the histological examination of the cellular content of the vaginal fluid. The rabbit is especially well adapted to such a study inasmuch as it readily gives evidence of hyperthyroidism following the ingestion of thyroid substances, and it can be completely thyroidectomized without showing symptoms of parathyroid deficiency.

METHOD AND EXPERIMENTAL PROCEDURE. We were unable to find more than citations of Pelkin's work; therefore our first problem was to determine the duration of time of the different phases of the sexual cycle in normal rabbits, and especially to determine the type of vaginal smear which was

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

indicative of the time when copulation occurred. Nineteen female rabbits of known laboratory stock, nutritional state, and of an age favorable for reproduction were examined daily by the vaginal smear method for approximately five months (Oct. 14, 1926, to March 21, 1927). In the rabbit it is quite possible to evert the lower half of the vagina and apply a clean microscopic slide directly to the vaginal mucosa, thus getting a sample of the cells in the lumen of the vagina with no danger of trauma as may occur when a spatula is employed in sampling. The specimen thus obtained on the slide is fixed, stained and examined microscopically. This technique requires the coöperation of two persons, one for firmly holding the rabbit (ventral side upwards), while the other everts the vagina and applies the slide.

The following variations in the experimental condition of the rabbits were introduced. October 14, 1926, to January 14, 1927, all were in normal condition. January 14, 1927, to March 21, 1927, nine were in a state of hyperthyroidism due to the daily ingestion of desiccated thyroids varying in amounts from 90 mgm. to 660 mgm. per day per rabbit. All thyroid-fed rabbits were treated until symptoms of toxicity became evident as indicated by tachycardia, loss in weight, increased respiratory rate and marked thirst. Two of the rabbits were totally thyroidectomized 28 months before these data was obtained and showed pronounced symptoms of myxedema (pot-belly, flabby muscles, loss of hair, scaly skin, apathetic appearance and less active than their normal litter mates). In addition to this, vaginal smears were made of nine rabbits through twelve different pregnancies.

Results and discussion. The daily data from these experiments were carefully compared from time to time. Space will not permit its entire publication; moreover this is unnecessary inasmuch as the results contained in table 1 are quite typical of the findings of all normal rabbits. Table 1 contains data taken from the protocols of three rabbits (animals I, IV, and XIV). The lack of cyclic variation in the appearance of the different types of epithelial cells or leucocytes is strikingly apparent; for example, in the case of animal I at one period (Dec. 22nd to Jan. 5th), leucocytes were present in great numbers for 15 consecutive days. At other times they appeared for periods of only 1, 2, 3, or 4 days. In animal IV the duration of the periods for which leucocytes were found varied from 1, 2, 5, 7, 9 and 11 days. In animal XIV the duration of the leucocytes varied over periods of 1, 2, 3, 4, and 5 days. During these periods either or all types of vaginal epithelial cells may be present in approximately equal numbers or either type may dominate the picture, but with a complete absence of regularity of appearance or disappearance so that periodic occurrence of either epithelial cells or leucocytes in the lumen of the vagina of rabbits cannot be demonstrated. This same statement also applies to the vaginal

TABLE 1

Showing daily variations in the histological examinations of vaginal smears of three rabbits over a period of three months. The number of leucocytes are indicated by +: the epithelial cells are classed into three groups; type 1 indicating the large thin flat cell with relatively small or no nucleus; and type 2, the medium sized squamous epithelial cell with a well-defined nucleus; and type 3, the small well-stained cell with a relatively large and deeply stained nucleus

DATE	LEUCOCYTES	TYPE OF CELLS	NUMBER OF CONSECUTIVE DAYS	DATE	LEUCOCYTES	TYPE OF CELLS	NUMBER OF CONSECUTIVE DAYS
Animal 1							
Oct., 1926, 14–16	+++	1–3	3	Nov., 1926, 25	0	2–3	1
Oct., 1926, 17–18	0	1	2	Nov., 1926, 26	+++	2–3–1	1
Oct., 1926, 19	+++	1–3	1	Nov., 1926, 27	+	2–3	1
Oct., 1926, 20–21	0	3	2	Nov., 1926, 28–29	+++	3–2	2
Oct., 1926, 22	+++	1	1	Nov., 1926, 30–Dec. 2	0	2–3–1	3
Oct., 1926, 23	+	1	1	Dec., 1926, 3	+++	2–3	1
Oct., 1926, 24–27	+++	3–2–1	4	Dec., 1926, 4	+	3–2	1
Oct., 1926, 28	0	1	1	Dec., 1926, 5–9	+++	1–2–3	5
Oct., 1926, 29–Nov. 1	+++	1–3	3	Dec., 1926, 10	0	3–1	1
Nov., 1926, 2	0	1	1	Dec., 1926, 11	+++	1–2	1
Nov., 1926. 3	+	1	1	Dec., 1926, 12	+	1–2	1
Nov., 1926, 4–6	+++	3–1	3	Dec., 1926, 13–15	+++	3–2	3
Nov., 1926, 7	+	3–1	1	Dec., 1926, 16	+	2	1
Nov., 1926, 8	+++	2	1	Dec., 1926, 17–20	+++	2–3–1	4
Nov., 1926, 9	0	1	1	Dec., 1926, 21	0	3	1
Nov., 1926, 10	+++	3	1	Dec., 1926, 22–Jan. 5	+++	3–2–1	15
Nov., 1926, 11	0	2	1	Jan., 1927, 6–7	0	1	2
Nov., 1926, 12–15	+++	3–2	4	Jan., 1927, 8	+++	1–3	1
Nov., 1926, 16–19	0	3–1–2	4	Jan., 1927, 9	+	1	1
Nov., 1926, 21–23	0	1–2–3	3	Jan., 1927, 10–13	+++	2–1	4
Nov., 1926, 24	+++	2–3	1	Jan., 1927, 14	0	1	1
Animal 4							
Oct., 1926, 14–15	+++	1	2	Nov., 1926, 29–30	+++	3–2	2
Oct., 1926, 16–22	0	1	7	Dec., 1926, 1	0	1	1
Oct., 1926, 23	+++	1	1	Dec., 1926, 2	+++	3–1	1
Oct., 1926, 24–Nov. 2	0	1	10	Dec., 1926, 3	0	3–1	1
Nov., 1926, 3	+	3–1	1	Dec., 1926, 4–10	+++	2–3	7
Nov., 1926, 4	0	1	1	Dec., 1926, 11	0	1	1
Nov., 1926, 5	+++	1	1	Dec., 1926, 12	+++	3–1	1
Nov., 1926, 6–7	0	1	2	Dec., 1926, 13	0	1–2	1
Nov., 1926, 8–9	+++	3–2–1	2	Dec., 1926, 14–15	+++	1	2
Nov., 1926, 10	+	1	1	Dec., 1926, 16	++	1–3	1

TABLE 1—*Concluded*

DATE	LEUCOCYTES	TYPE OF CELLS	NUMBER OF CONSECUTIVE DAYS	DATE	LEUCOCYTES	TYPE OF CELLS	NUMBER OF CONSECUTIVE DAYS
Animal 4—*Concluded*							
Nov., 1926, 11–14	0	1	4	Dec., 1926, 17–27	+++	1–2–3	11
Nov., 1926, 15	+++	2–1	1	Dec., 1926, 28	0	1	1
Nov., 1926, 16–19	0	1–2	4	Dec., 1926, 29–Jan. 6	+++	1–2–3	9
Nov., 1926, 20–21	+++	2–3	2	Jan., 1926, 7	0	2–3	1
Nov., 1926, 22–23	0	1–3–2	2	Jan., 1927, 8–12	+++	2–1–3	5
Nov., 1926, 24	+++	2	1	Jan., 1927, 13–14	+	1–2	2
Nov. 1926, 25–28	0	2–1	4				
Animal 14							
Oct., 1926, 14	+	1	1	Dec., 1926, 1	+++	2–3	1
Oct., 1926, 15–19	0	3–1	5	Dec., 1926, 2–3	0	1–2–3	2
Oct., 1926, 20	+	1–3	1	Dec., 1926, 4	+++	2–3	1
Oct., 1926, 21	+++	2	1	Dec., 1926, 5	0	1	1
Oct., 1926, 22–29	0	1–2	8	Dec., 1926, 6	+++	1–3	1
Oct., 1926, 30–Nov. 1	+++	1	3	Dec., 1926, 7–9	0	1	3
Nov., 1926, 2	0	1	1	Dec., 1926, 10	+++	1–3	1
Nov., 1926, 3	+++	1–2	1	Dec., 1926, 11–14	0	1–2	4
Nov., 1926, 4	0	1	1	Dec., 1926, 15–16	+++	1	2
Nov., 1926, 5	+	1	1	Dec., 1926, 17–21	0	1	5
Nov., 1926, 6	0	1–2	1	Dec., 1926, 22–23	+++	1–3	2
Nov., 1926, 7–11	+++	1–2–3	5	Dec., 1926, 24–25	0	1–3	2
Nov., 1926, 12	0	1	1	Dec., 1926, 26–27	+++	2–1	2
Nov., 1926, 13–17	+++	1–2–3	5	Dec., 1926, 28–31	0	1–2	4
Nov., 1926, 18	0	1	1	Jan., 1927, 1	+++	1	1
Nov., 1926, 19–21	+++	1–2–3	3	Jan., 1927, 2–5	0	1	4
Nov., 1926, 22–23	0	1	2	Jan., 1927, 6	+++	1–2–3	1
Nov., 1926, 24–26	+++	1–2–3	3	Jan., 1927, 7–8	0	1	2
Nov., 1926, 27	0	1	1	Jan., 1927, 9	+++	1	1
Nov., 1926, 28–29	+++	1–2–3	2	Jan., 1927, 10–11	0	1–2	2
Nov., 1926, 30	0	1	1	Jan., 1927, 12–13	+++	1–2	2
				Jan., 1927, 14	0	1	1

smears of rabbits obtained during pregnancy, and during periods of induced hyperthyroidism. The two rabbits with myxedema showed unusually long periods during which the vaginal fluid contained no leucocytes (longest period 18 days). During these periods the epithelial cells were much fewer in number than occurred in the normal rabbits. These rabbits never became pregnant in the myxedematous state but subsequent to the feeding

of thyroid substance both animals delivered litters of apparently normal young.

The absence of periodic recurrence of a characteristic cell type in the lumen of the vagina of rabbits at regular intervals is a striking contrast to the phenomena demonstrated by Evans and Long (1922) in the rat and

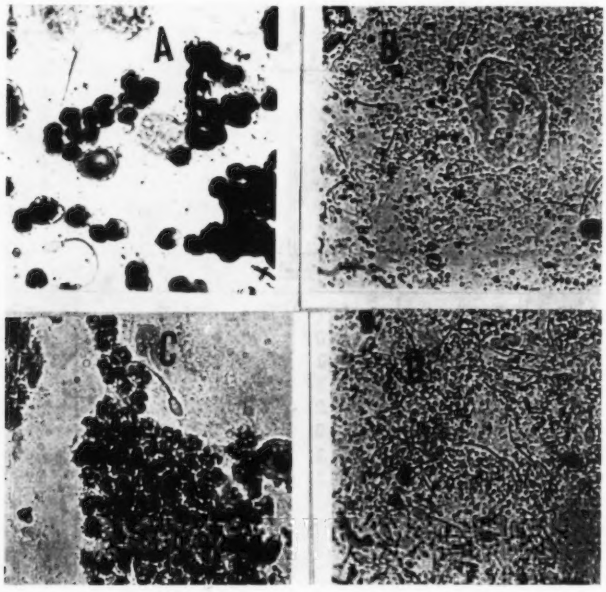

Fig. 1. Photomicrographs of vaginal smears showing that copulation in the rabbit occurs in the absence of characteristic cells in the lumen of the vagina. A. shows sperm among many round epithelial cells, uniform in appearance and size and usually present in sheets. These cells have relatively large deeply stained nuclei. B. shows sperm with no leucocytes and only a few large flat thin epithelial cells with very small or no nucleus. D. shows only sperm and deeply staining granular substances present. C. shows sperm among myriads of leucocytes and medium sized nucleated epithelial cells. (Mag. X 233.) These smears were taken 15 to 60 minutes after copulation. The histological elements in these smears ane identical (except for the sperm) with those taken immediately before copulation.

confirmed by many other workers. These authors predict with exactitude from a histological study of the vaginal contents, the exact time when copulation will occur. The dioestrus interval engages about one-half of the entire cycle and the oestrus is inaugurated by proestrum (stage 1) characterized by no leucocytes and a great number of small round nucleated epithelial cells of uniform appearance and size, occasionally in small sheets.

The average duration of this stage is 12 hours. At the beginning of this period no mating occurs but may occur during the latter half. Stage II shows large thin transparent non-nucleated scale elements; these are the cornified cells. No leucocytes are present and these findings are an unmistakable sign of heat. Stage III shows the same signs as above, but rapid accumulation of cornified cells and masses of whitish granular substance in the vagina. No coitus occurs during this stage. The duration of stages II and III is about 30 hours. Stage IV is only 6 hours long. Now leucocytes appear amid cornified cells and epithelial cells (not in groups) reappear, and so for a short time all of the cellular elements are present until dioestrum is ushered in and the cornified cells completely disappear, leaving only leucocytes and epithelial cells present.

That this well-defined demarcation of stages which allows one to predict the exact time when copulation in the rat occurs, cannot be demonstrated in the rabbit is clearly shown by a series of studies made in nine females not included in the group used for the daily study of vaginal smears. These nine rabbits were isolated in separated cages excepting for short periods each day when they were placed with a male and kept under vigilance. Vaginal smears were made of each female before placing with the male and watched for one hour unless copulation occurred at once. If mating did occur vaginal smears were again made within fifteen minutes and the females replaced in the isolation cage until after the young were born. If no copulation occurred within an hour, they were reisolated and the process repeated daily until vaginal smears containing sperm were obtained from each of the 9 females. Females thus observed delivered young from 29 to 31 days after the observed copulation.

Figure 1 (A, B, C, D) shows microphotographs of vaginal smears of four of these rabbits made immediately after copulation and with sperm on the smears. A shows sperm in the presence of no leucocytes but many small round nucleated epithelial cells of uniform appearance and size, usually present in sheets (arbitrarily called type 3 cells). This seems to correspond to the description of proestrum in the rat. B shows masses of sperm with no leucocytes and large thin non-nucleated or cells with small nuclei. This type of cell probably corresponds to the cornified cells found in stage II of the rat, but in the rabbit typical cornified cells are rarely seen. D shows nothing but granular bluish staining material with the sperm and were it not for the absence of many cornified cells might correspond to the rat III stage. C shows sperm among myriads of leucocytes (theories regulating the occurrence of leucocytes have been discussed by Lehman (1921) and Corner (1923)) and medium-sized nucleated epithelial cells (type 2) as occurs in dioestrum in the rat. These illustrations are sufficient to demonstrate that copulation in the rabbit occurs when the cellular content of the lumen of the vagina of rabbits is typical of either of the cyclic changes described for the rat.

The cycle in the guinea pig differs somewhat from that described for the rat. Papanicolaou and Stockard showed that ovulation occurred during the second or third stages; the second stage characterized by enormous numbers of epithelial cells with well-stained nuclei; the third stage, serous-like fluid and great numbers of polymorphonuclear leucocytes with smaller numbers of epithelial cells.

Hartman (1923) studying the opossum seems to indicate that the clear-cut picture of the different phases of the normal cycle is dependent upon the progress of a perfectly developing follicle. This author states that if the follicle degenerates while medium sized or smaller, the vaginal smear picture becomes obscure and there is a continuous dioestrus smear containing nucleated epithelial cells, but if the follicles reach maturity the vaginal changes are more or less clear-cut and involution of the vagina proceeds whether the follicle ruptures or not. This stresses the importance of atresia of the follicles in connection with vaginal smear studies. Some histological evidence which we have at hand seems to indicate that atresia of the follicles frequently occurs in rabbits which in the light of Hartman's work strongly suggests that this may be the cause of the obscure pictures found in the vaginal smears of rabbits. More evidence is needed on this point. Other contributing factors may be the absence of spontaneous ovulation and the rare occurrence of typical cornified cells.

Marked changes in the degree of congestion of the external genitalia were observed from time to time, but this is by no means a reliable sign as it may persist throughout an entire gestation period. Allen (1922) noticed similar discrepancies in his studies of the mouse.

SUMMARY

1. Copulation and fertilization in the rabbit occur in the presence of a cellular content of the lumen of the vagina, comparable to either of the cyclic changes described for the rat.

2. No regularity in the appearance or disappearance of the different types of epithelial cells or leucocytes, in the lumen of the vagina, was observed in normal rabbits. This also applies to observations on thyroidectomized rabbits, pregnant rabbits and rabbits with induced hyperthyroidism.

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THE INFLUENCE OF THE PROPRIOCEPTIVE SYSTEM UPON THE CROSSED EXTENSOR REFLEX[1]

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Contralateral reflexes have for many years been an object of enquiry. They were first observed in decerebrate animals (1898) by Sherrington (14), and investigations since that time have lately been summarized by several authors (6), (7), (17), (2). Reflex responses originating within the body, especially those arising from the action of gravity, or from change of position in space, have been termed "proprioceptive" (15), (16). The chief receptive fields for this group of reflexes are: 1, the labyrinth; 2, the muscles of the neck; 3, the skeletal muscles. The present communication will concern itself chiefly with the influence of groups 2 and 3 upon the crossed extensor reflex. The importance of proprioceptive reflexes has often been emphasized, especially by the Spanish workers who have so effectively elucidated the morphology (e.g., 22) of the proprioceptive end-organs. A. Pi-Suñer has referred to these end-organs very happily as the "starting-point of reflex integration" (11).

Previous observations. A muscle such as the quadriceps (cat) contains at least two different functional groups of afferent end-organs (18, p. 210); (8, p. 239); (2, pp. 379 and 431), for, "though the knee-extensor when subjected to a stretch responds by a proprioceptive reflex *contraction*, faradisation [e.g., by a single shock] of the central end of cut branches of its own nerve produces reflex *inhibitory* relaxation of the muscle." Evidence has been presented elsewhere (2, ch. xvii) to show that both types of proprioceptor influence may be observed during a normal crossed extensor reflex, the inhibitory efferents operating during the ascent of the reflex, and the excitatory ("stretch") afferents being often stimulated during relaxation. The proprioceptive inhibitors are set into activity by *sudden onset of contraction*, in this way preventing undue rise of internal stress, and they are in part responsible for the S-shaped appearance so often observed in the ascending curve of the crossed extensor reflex (fig. 1) and

[1] The substance of this paper was communicated at Rochester, N. Y., April 14, 1927, at the meeting of the American Physiological Society under the title of: "Observations upon temporal summation and upon inhibition of the crossed extensor reflex before and after deafferentation."

in the knee-jerk for the "silent period" in the electrical record which ensues immediately upon the rise of tension (Denny-Brown, 1; cf. 12).

In the experiments which we are about to describe, the crossed extensor reflex was evoked by employing two or three single break shocks following each other at varying intervals. In this way the response to each successive stimulus could be studied in detail, and satisfactory analysis could be secured of the several proprioceptive influences which may be brought to bear upon the response. Incidentally we have secured by this method further information concerning the problem of the *addition latente* of this reflex (see 20). In early experiments our preparations showed great variability in the ease of elicitation of crossed extensor responses by single break induction shocks. In some animals the reflex could not be obtained at all, while in others the response to a single contralateral stimulus was vigorous. Subsequent observations indicated that this variability in response was due in part to the position of the head, which in our first experiments was not adequately taken into account.

METHOD. Cats were decerebrated at the mid-collicular level by the trephine method under profound ether anesthesia. All nerves in both hind limbs were cut with the exception of the nerve to the quadriceps muscle on one side, in accordance with the method described by Sherrington (16). The branch of the hamstring nerve passing to the semitendinosus muscle was permitted to remain intact when we wished to study flexor responses simultaneously with those of the extensors. Precautions were taken to exclude activity of the tensor faciae femoris by liberal resection. The cord was exposed in the lumbar region under the original anesthetic. After observations were obtained the preparation was again anesthetized and appropriate posterior roots were then sectioned intradurally. The central end of the sciatic nerve trunk was used for elicitation of both the ipsilateral and the contralateral reflexes.

The optical isometric technique recently described (2), involving the use of the Sherrington torsion-wire lever of high natural vibration frequency (800–1200) in the same optical system with a string galvanometer (Cambridge, Einthoven), has been used throughout. We are greatly indebted to Mr. J. H. Emerson who has considerably modified the myograph for us in order to permit the greater magnification of its shadow occasioned by the introduction of the myograph point into a projection ocular (Zeiss no. 6) used on the galvanometer. In place of a needle ground to a fine point, Mr. Emerson has substituted a light-tempered brass carrier with two terminal horizontal arms across which is stretched (vertically) a mouse hair 10μ in diameter. Quartz fibres have also been employed. The use of a projection ocular in place of the focussing lens, for bringing the myograph into the optical system of the galvanometer, has greatly improved the optics of the recording system, and we wish to express our

gratitude to Prof. Alexander Samojloff of Kasan for suggesting its use. A double myograph built on this plan has also been devised by Mr. Emerson to enable simultaneous registration of responses of flexors and extensors. This has been used in some of our more recent experiments.

MUSCLE PROPRIOCEPTORS. Under constant conditions of the neck and labyrinthine proprioceptive fields, interruption of the proprioceptive impulses from the quadriceps muscle, by appropriate posterior root section, alters 1, the threshold; 2, latency; 3, magnitude; 4, duration (after-discharge); 5, the process of central summation, and 6, the susceptibility to ipsilateral inhibition of a contralateral reflex set up by a single break induction shock. We may consider these alterations in the order mentioned. For purposes of exposition we shall refer to preparations with posterior roots intact as "normal" and to those with posterior roots cut as "deafferented."

Threshold. In the majority of preparations (and apparently in all in which the cord was not traumatized by exposure of the roots) the threshold for the reflex was higher before posterior root section than after. Thus, from a Harvard coreless coil whose primary was fed by a current of 0.1 ampere, a stimulus at 3 to 4 cm. coil distance of the secondary was necessary in one experiment to produce a response, while after posterior root section a break shock with the coil at 8 to 9 cm. coil distance produced a response of the same magnitude, the muscle being at the same initial tension. In other preparations even larger differences were noted (e.g., fig. 1A and 1B).

Latency. The latency of the crossed extensor reflex is notoriously variable (19), and this is undoubtedly to be associated with the process of recruitment which characterizes the reaction (6). That is, with mere continuance of an otherwise unaltered *tetanic* stimulus increasing numbers of neuromuscular units are brought successively into activity (7), and recruitment is evidently a visible continuation in the reflex contraction of what precedes it invisibly in the latent period; i.e., an *addition latente* (20). But with the use of single stimuli an *addition latente* associated with the terminations of the afferent neurones can be excluded. At some point or points in the passage of the volley of impulses across the cord, however, a central process is set up which leads to repetitive responses in the anterior horn cells subserving the vastocrureus muscle (see *Duration* below for evidence of repetitive response). Since repetitive discharge implies temporal summation, it is evident that an *addition latente* may even be a feature of the central process set up by a single break shock. We have occasionally observed diminution in the latency of the reflex with increase of stimulus intensity. At a coil distance of 5 cm. the latency was 65σ while at 2 cm. in the same preparation it was only 40σ. The increased number of fibres brought into action by the stronger stimulus has served to increase the

"size" of the central excitatory process (19; also 2, ch. xiv) and so to diminish the latency of the reflex.

After deafferentation, other things remaining constant, the latency of the reflex is often *less* than before but this is not invariable (cf. fig. 1). This was noted by Fulton and Liddell (3, p. 217) for tetanic responses, and

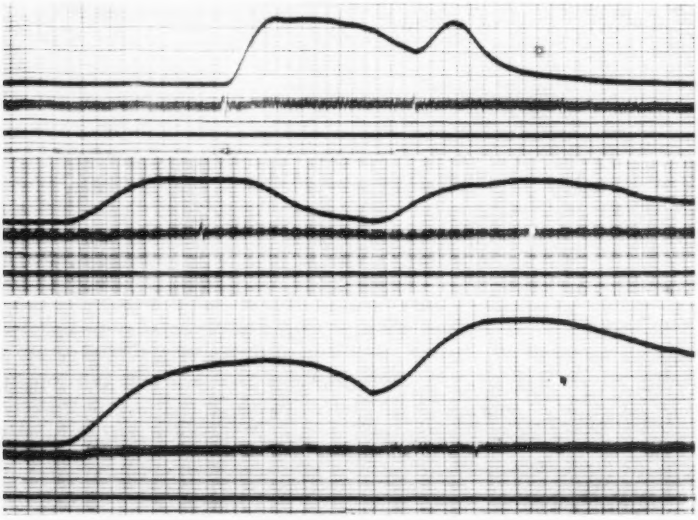

Fig. 1. Crossed extensor reflexes to single break shocks. Moment of stimulation indicated by signal lines at the bottom of each figure. Vertical coördinates (in all figures) give time in 0.02 second; horizontal divisions represent millimeters on original plate: 52 divisions = 1 kilo tension; line of zero tension above lowest signal.

A. Quadriceps undeafferented and head normal. Responses are shown to two equally intense break shocks (coil distance 1 cm.) applied to contralateral sciatic at an interval of 305 σ. Note small size of second response. (There was no response to a single stimulus with the coil at 5 cm.)

B. Same preparation one hour after deafferentation with head normal. The responses are shown with the coil at 5 cm. and stimulation interval of 505 σ. With stimuli closer together (*e.g.*, as in A), the second response was still larger and more prolonged.

C. Same as B with head rotated (chin toward recording muscle). Note great increase in size of responses

the same phenomenon has again been observed in response to single break shocks. Latencies of 60 and 65σ before posterior-root section have become reduced to 40 to 50σ. In some preparations even greater differences were noted (50 to 25σ).

Magnitude. The increase in size of the crossed extensor response to

a single break shock in a recently deafferented preparation over that observed in a "normal" animal, is indeed striking. With the same stimulus intensity and conditions of initial tension, etc., a "normal" muscle may develop only 100 to 200 grams tension, while after deafferentation it may yield a response of nearly a kilo.

Duration (*after-discharge*). Though occasionally the duration of response may be approximately the same, usually after deafferentation the response lasts considerably longer, as measured to the termination of the tension plateau, than before deafferentation (fig. 1A and 1B). The duration of the response, if compared with that of a single motor nerve twitch of the same muscle is found to exceed it by three of four hundred per cent, or often more, depending upon the position of the neck. This shows unequivocally that the reflex response is tetanic in character, and occasionally when the "angle" is sharp, indicates that the majority of fibres have responded the same number of times.

Summation. To study the process of summation, two (or sometimes three) equally intense break induction shocks were applied to the contralateral sciatic nerve at varying intervals. In "normal" preparations when the interval between two such successive stimuli was equal to or briefer than the latent period of the reflex, the resulting tension developed in the fused response was several hundred per cent greater than that produced by a single stimulus. This indicates that the second stimulus activated a large number of elements which, though inaccessible to the first stimulus alone, were by it rendered accessible to the second stimulus. This we take to be a manifestation of the *addition latente* underlying the process of recruitment. When the second stimulus occurred during the ascent of response I, response II was less ample than before, and if stimulus II occurred during the early stages of relaxation of response I, response II was usually smaller still, showing little or no evidence of recruitment (fig. 1A). Presumably the active contraction resulting from the first stimulus initiated proprioceptive impulses which reflexly inhibited further activity in the muscle. This effect has been referred to as "autogenous inhibition" (4), (2).

There are, however, certain reservations to the last statement, for in some instances the diminished size of the second response was not evident. This was the case with very weak stimuli in which the first response was ordinarily small and of slow onset. Presumably under these circumstances proprioceptive inhibitors are not stimulated. When, however, the first response was ample and of rapid onset, the effect of which we have just spoken was always present. Why certain preparations, under constant conditions of the neck musculature, yield large responses to single contralateral break-shock stimuli and others small ones, is not yet evident, though it is possibly associated with nutritive conditions and with varying degrees of "shock" incidental to operative procedures, depth of operative anesthesia, and variable factors of this type. Our most constant results were obtained in preparations in

which the original anesthetic lasted not longer than 30 minutes. Preliminary observations upon animals which have been permitted to survive for 24 hours or more following decerebration also suggest that shock may be the cause of the variations observed in acute preparations, for after 24 hours a single break shock apparently always yields an ample response.

It may be mentioned also that since the outstanding characteristic of a crossed extensor reflex is recruitment, the size of the second response would tend in the absence of inhibitory influence to be larger than the first. If, therefore, it becomes equal in size to the first response, the result is evidently due to proprioceptive inhibition. Usually in normal preparations the duration of the second response is markedly curtailed as contrasted with the first (fig. 1A). This is very characteristic and is clearly due to the muscle's proprioceptors, for we have never observed it after posterior root section.

After deafferentation we have been unable to demonstrate diminution in size or duration of a second response when it falls at any point during or after a first response. On the contrary, the recruitment process is always in evidence, response II being larger than response I (fig. 1B and 1C) and sometimes larger by several hundred per cent. Response II reaches its maximum size and duration when it falls within a few σ after stimulus I. It becomes progressively smaller as the time-interval from the beginning of the first stimulus is made greater. Variation of interval between the first and second stimuli, therefore, forms a valuable method of investigating the rate of dissipation of the excitatory process by a given first stimulus.

Approximately two seconds must elapse before a second response becomes equal in size to a first response. The first stimulus, that is, renders certain neurones which it did not actually excite accessible to a second stimulus for a period of two seconds. This emphasizes the prolonged character of the central excitatory process (see also Sherrington, 19).

Susceptibility to inhibition. By appropriate posterior root section it is possible in most cats completely to deafferent the quadriceps muscle while leaving still intact the posterior root supply of a portion of the sciatic and the hamstring nerves. Consequently the susceptibility of the crossed extensor reflex to ipsilateral inhibition may be tested both before and after deafferentation. In our experience a single break-shock stimulus applied to the ipsilateral sciatic nerve is as effective in producing inhibition before as it is after section of the afferents to quadriceps. In two preparations such a stimulus was definitely more effective despite the smaller number of available posterior roots through which the impulses would be carried to the cord. Inhibition of the crossed extensor reflex by means of flexor traction appeared quite definitely to be more readily obtained after deafferentation of the quadriceps than before, but as this is a matter in which subjective impressions cannot well be excluded the measurements with

single break-shock stimuli are undoubtedly more satisfactory. The evidence thus far available therefore suggests that a muscle deprived of its afferent nerve supply is more readily inhibited than a normal muscle.

THE NECK REFLEXES. Soon after the present research was undertaken it was found that the ease with which single break-shock stimuli produced crossed extensor responses was largely conditioned by the position of the head. This indeed might have been predicted from the investigations of Magnus and de Kleyn (9), (10) and their collaborators (see especially Socin and Storm van Leeuwen, 21) upon the tonic neck and labyrinthine reflexes. When labyrinthine influence is excluded they have found that twisting of the head causes augmentation of extensor tonus on the side toward which the chin is rotated, and diminution of tonus on the opposite side. Similarly, when the neck reflexes are excluded, placing the animal in the supine position causes maximum extensor tonus in all four limbs. In our experiments the head was rotated in such a way as to produce an augmentation in tonus both from labyrinthine and neck proprioceptive fields. In these observations we did not distinguish between the neck and the labyrinthine influence.

In our hands the influence of rotation of the head upon the crossed extensor reflex can be demonstrated both in the normal and the deafferented quadriceps muscle (cat). Sherrington (17, p. 199) and Magnus (9, p. 70) have observed that the tonus of the quadriceps can be influenced similarly, deafferentation having but little effect upon the response. There is in fact little difference in the response in the two conditions unless it be that the duration of the response is less easily influenced in the normal preparation by twisting of the neck than in the deafferented (see below).

It has been our general impression, moreover, that after deafferentation of the quadriceps the muscle becomes more sensitive to tonic neck and labyrinthine influence than it was before. In several preparations we have attempted to measure the increase in sensitivity by observations upon the degrees of neck rotation. In one before deafferentation, deviating the chin approximately 70 degrees from the midline was required to produce a well-marked effect, while after deafferentation deviation of 45 degrees produced the same effect.

We propose in the discussion which follows to confine the description to the influence of neck rotation upon the *deafferented* muscle, since under these circumstances there are fewer complicating factors. It will be useful to preserve the same headings as were used in the preceding account of the muscle proprioceptors, for rotation of the head has been found to alter: 1, the threshold; 2, latency; 3, magnitude; 4, duration; 5, the process of central summation, and 6, the susceptibility to ipsilateral inhibition of an extensor reflex set up by a single contralateral break induction shock.

Threshold. Twisting of the head of a deafferented preparation (chin

toward the recording muscle) may diminish the threshold from 0 cm. coil distance to 12 cm. for a single break induction shock from a Harvard coil with primary fed by 0.1 ampere. The alteration in threshold is indeed one of the most striking features of the tonic neck and labyrinthine influence. The threshold, broadly speaking, becomes altered *pari passu* with gradual rotation of the neck, but there appears to be a critical angle when the neck is at about 90 degrees from its resting position, when the threshold falls with great abruptness.

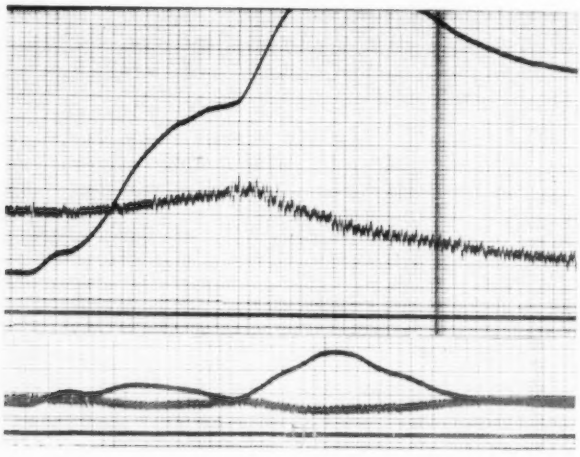

Fig. 2. Crossed extensor reflex; quadriceps undeafferented and responding to two equal break induction shocks at an interval of 385 σ; 38 horizontal divisions = 1 kilo; time = 0.02 second.

A. With head rotated *c.* 90° toward the recording muscle.

B. With head normal.

Note the differing magnitude and latencies and the S-shaped ascent of the first response.

Latency. Equally striking is the alteration in latency produced by twisting of the head (fig. 2). The following are random measurements from several preparations: 90σ with head normal, 50σ with head rotated (chin toward recording muscle); 60σ to 40σ; 85σ to 55σ; 65σ to 25σ, etc. With the second of two responses the diminution in latency produced by twisting of the head may be very striking indeed, e.g., a second response having a latency of 65σ was reduced on turning the head to 15σ. The briefest latencies observed in this reflex are those of the deafferented muscle with head turned, responding to second and third equal stimuli of a series. In one instance, under these circumstances, we observed a latency lying

between 11σ to 12σ, which is but 4σ to 5σ greater than the latency of the knee jerk.

Magnitude. The alterations in magnitude produced by twisting of the head are, relatively speaking, enormous (fig. 2). In certain preparations a single break shock with the head in the normal position, evoked a reflex in which 300 grams tension was developed. On turning the chin toward the recording muscle the tension development in successive observations lay between 1200 and 2000 grams. Usually, however, the variations are of the order of 100 per cent, a response of 250 grams becoming of the order of 500 to 600 grams with head rotation.

Duration. On rotating the chin toward the recording muscle, single break-shock reflexes produce not only a response of greater amplitude than before but one of very much greater duration (fig. 2), and this is an effect which apparently involves the majority of motor neurones, for the "angle" (2, ch. iii) is sometimes moderately sharp, denoting approximately synchronous cessation of activity among participating units. Thus, in the response shown in figure 1C, with the head normal the duration was 280σ, and with head left 360σ. In the same preparation a response of 340σ became 440σ upon turning the head, and one of 220σ, 360σ, etc.

The process of central summation. The process of central summation is similar in general with and without the head turned (see above). With the head turned, however, not infrequently response II, when it falls during the relaxation of response I, is of shorter duration than the first response. This effect was never seen when the head was in the normal position, and suggests some complex long-circuiting (2, ch. xxi) effect set up by single stimuli when the head is turned. Despite the curtailment of duration which sometimes occurs in the response to the second stimuli, the recruitment process, as judged by the increasing amplitude of second and third responses, still remains a constant feature of the reflex.

Susceptibility to ipsilateral inhibition; reflex reversal. The problem of the susceptibility to inhibition, when considered in relation to the neck reflexes, assumes a somewhat special aspect, for not only is the susceptibility greatly altered but actual reversal of inhibitory into excitatory effect may take place. The influence of the tonic neck and labyrinthine reflexes upon the response of extensor muscles was first investigated by Socin and Storm van Leeuwen (21). They pointed out that rotation of the head caused the response of the cat's triceps muscle (forelimb) to stimulation of its ipsilateral radial nerve to be converted from inhibition into excitation. Under constant conditions of the neck musculature similar "reversals" were also obtained by variation of intensity of stimulation. Sassa (13) mentions that in his experiments on constant current stimulation, altering the position of the head reversed the response of the quadriceps to ipsilateral stimulation. This author, however, did not investigate the problem in

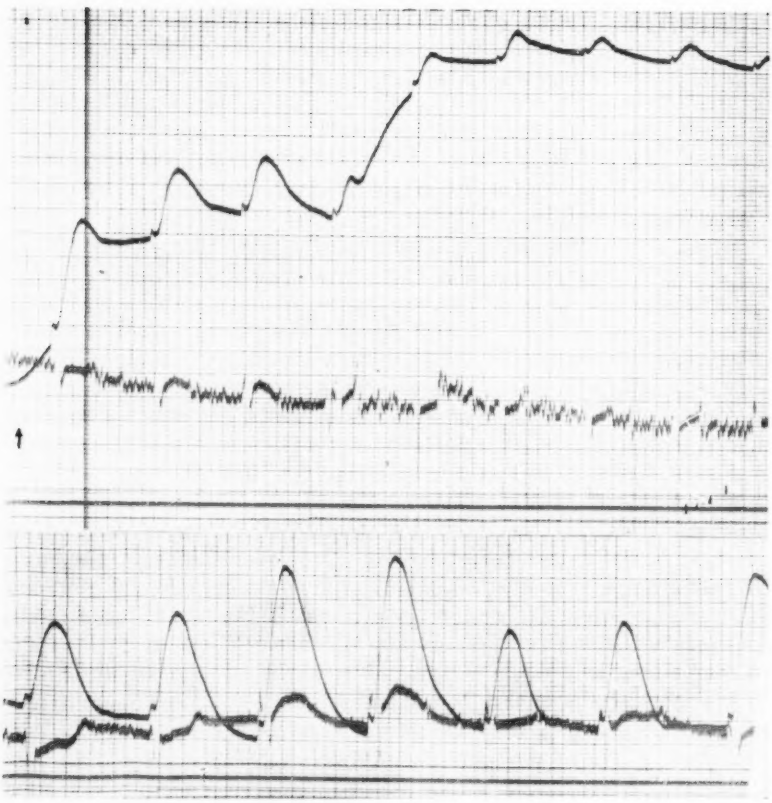

Fig. 3. A. Quadriceps undeafferented; at a point on the left of the record (see arrow) the head of the preparation was rotated with a resulting "spontaneous" increase in the postural contraction shown in the first half of the record; at a second point shown by the signal at the bottom of the plate the *ipsilateral* sciatic was stimulated weakly by a single shock and the resting contraction became *increased* still further after a brief inhibitory notch. Note the change in the knee-jerks which were delivered during the record.

B. Same with head normal. The single shock, also weak and applied to the ipsilateral sciatic, here causes inhibition of the resting contraction (the inequality of the knee-jerks here is due to unequal taps); 38 horizontal divisions = 1 kilo; time = 0.02 second.

detail. Girndt (5), in his analysis of the reflex of thalamic animals, has dealt extensively with the reversals in the responses of both flexors and extensors (hind limb) produced by the neck and labyrinthine reflexes.

Being unacquainted at the time with these observations, we were indeed surprised to find that four hours after decerebration the response of the quadriceps muscle to a single break-shock stimulus applied to the ipsilateral sciatic nerve was strikingly reversed by rotation of the head (fig. 3). Thus, when the head was in its normal position or when rotated *away* from the recording muscle ("minimal" position), a single break shock produced an inhibition of any "tonus" which at the time existed in the muscle, this being the usual response obtained under ordinary experimental conditions. When the chin was rotated toward the recording muscle, a break shock of the same intensity applied to the same nerve under otherwise identical conditions, produced a striking augmentation of "tonus," such as that shown in figure 3. Similar reversals were found to occur in the responses of the hind limb flexors.

Ipsilateral and contralateral reflex contractions of the quadriceps contrasted. Since, with rotation of the head toward the side of the limb under observation, vigorous extensor responses are to be obtained from break shocks applied to the ipsilateral nerves, it is of interest to compare the responses obtained from the respective sides. Markedly different are the *latencies* of the two. The ipsilateral extensor response usually does not occur until 50 to 120σ after the application of the stimulus, while (as we have seen above) the contralateral response with head turned may occur within 15 to 40σ. This suggests (but does not prove, see 2, p. 320) that the ipsilateral reflex is mediated through more devious reflex pathways than the contralateral reflex. The responses differ markedly also in their *duration*, the ipsilateral being prolonged and "tonic" in character, often lasting several seconds or more, while the contralateral seldom lasts longer than 300 to 400σ. This again suggests a different underlying mechanism. Perhaps more suggestive of a fundamental difference between the two is the fact that the ipsilateral is not infrequently preceded by a slight inhibitory relaxation (a "notch," see fig. 3A), and this is prone to occur when the head is *not* maximally rotated. Moreover, knee-jerks may be inhibited or become greatly diminished in size *without inhibiting the underlying "tonic" response.* The knee-jerk in fact may become greatly diminished even though it occurs during the ascent of the extensor response evoked by the ipsilateral stimulus. Thus in figure 3A the knee-jerks occurring during the initial tonic response caused by rotation of the neck are large, while those occurring during the second "tonic" response (evoked by the ipsilateral break shock) are small. Particularly striking is the contrast between the first and the fourth knee-jerks. Note also that beginning at a point 30σ after the break shock is a brief inhibitory "notch" lasting about

12σ, it being interrupted by the tap for the jerk. By interpolation the beginning of the tension-increase of the ipsilateral reflex occurs about 70σ after the break shock, but this of course cannot be determined with accuracy on account of the jerk.

In emphasizing that the knee-jerk is apparently inhibitable under these circumstances independently of the "tonic" substratum, we do not wish to have it inferred that a different peripheral mechanism exists for the knee-jerk and for the so-called "tonic substratum." There is no well-supported justification for such an inference in the present state of knowledge. What the phenomenon obviously suggests is some difference in the central paths of the two reflex effects. The long latency of the ipsilateral response naturally leads one to suppose that this reflex effect depends upon some distant center, possibly in the medulla, while the inhibitory effect, being immediate,[2] is clearly mediated through spinal centers (see 2, p. 323). The differing time relations of these two reflexes would in themselves be sufficient to account for their independence. The phenomenon is, moreover, quite compatible with the schema of reflex processes set forth elsewhere (2, p. 534), which rests on the assumption that all types of active contraction in skeletal muscle of vertebrates are produced by one peripheral mechanism.

The central paths for the contralateral extensor reflex are probably entirely spinal, since, after recovery from shock, spinal animals exhibit crossed reflexes (Sherrington, 16). The latency (e.g., $12–15\sigma$; *v. sup.*) observed for second responses makes this even more likely.

The neck reflexes and reversal of response of the hind limb flexors. Since rotation of the head causes a reversal in the response of the quadriceps extensor muscle to a stimulus applied to the ipsilateral sciatic nerve, it is interesting to examine the influence of head rotation upon the response of a knee flexor, such as semitendinosus; for, if reciprocal innervation holds, one would anticipate with neck rotation an effect opposite in the flexors to that observed in the extensors. This we have examined by using a double optical myograph which records simultaneously the responses of the flexors and extensors. Electrical responses have been obtained from one or the other of the two muscles, but we have not yet succeeded in obtaining simultaneous electrical records of the two.

With the head normal, stimulation of the *ipsilateral* sciatic caused a

[2] Note that the inhibitory notch begins $30\ \sigma$ after the break shock. This, as has been pointed out elsewhere (2, p. 323), is the least interval at which an inhibitory stimulus can possibly diminish a pre-existing reflex, for even if the impulses traversing the motor nerve were instantaneously abolished the muscle would remain in a state of tension for 25 to $30\ \sigma$ on account of the duration of its twitch contraction. This fact is often lost sight of in considerations of the relative latencies of inhibitory and excitatory reflexes (*e.g.*, in reciprocal innervation).

large flexor response and inhibition of extensor tonus, while with the neck rotated the flexor response became diminished almost to nothing and the extensor contracted instead of being inhibited. Moreover, when a single stimulus was applied to the *contralateral* nerve a large flexor response occurred if the chin were rotated away from the recording muscle. The extensor did not respond at all or was sometimes even inhibited. When the head was rotated toward the recording muscle the extensor response to a single contralateral stimulus was large (see above) and occasionally there was an actual inhibition of the flexor if at the time of stimulation there was any flexor contraction. These observations are in harmony with those of Girndt (5).

From these experimental results it would appear obvious that it is improper to speak of an "inhibitory" stimulus, as is the common usage with reference to an ipsilateral stimulus and its effect upon the quadriceps extensor muscle, for the reflex result of such a stimulus is conditioned by the position of the neck muscles.

Discussion. Our observations recorded in part I confirm a previous inference (2, p. 437) that proprioceptive inhibitors exist within the quadriceps extensor and that normally these endings are called into activity by active contraction. When tension is developed too rapidly the muscle inhibits itself (autogenous inhibition) and in this way prevents undue internal stress. The value to the organism of such a protective reflex is obvious. Through it is also gained smoothness of response. Normal extensor reflexes do not begin *en masse*, but operate gradually and smoothly even with vigorous effort.

Our purpose in studying the neck reflexes, however, was not to elucidate their significance to the organism, for this has already been done by Magnus (9) and his collaborators with a completeness that commands the admiration of all careful investigators. We have sought rather to investigate the intimate nature of the mechanism by which the neck and labyrinthine proprioceptors influence the spinal centers. Three points are clear.

1. Since the latency of the crossed extensor reflex becomes diminished when the head is appropriately rotated and since the reflex response becomes at the same time larger, it is evident that some impediment which previously hindered the passage of impulses across the cord is removed by rotation of the head. It is in harmony with the well-recognized inhibitory activity of the higher centers of the nervous system upon the lower to suppose that the impediment is due to some form of continuous inhibitory stimulation.

2. Now the "release" which facilitates the passage of impulses from one side of the spinal cord to the other also converts the ipsilateral extensor response from one predominantly inhibitory to one predominantly excita-

tory in its reflex effect. The latency of the ipsilateral excitatory response suggests, as mentioned above, the direct participation of some higher center or a response involving "long-circuiting" (2, p. 531). Evidently, therefore, head rotation not only facilitates contralateral stimulation but it appears to render certain of the higher centers more accessible to ipsilateral stimulation than when the head is normal. In short, one is tempted to believe that reflex "reversal" is brought about through activation of some higher center of the nervous system rather than through the intrinsic activity of the spinal centers.

3. Finally, since the flexors respond to the neck reflexes in exactly the opposite way to the extensors, it is evident that the neck reflexes are mediated through a mechanism involving reciprocal innervation.

It is perhaps of interest to point out that when the head is in "minimal" position the restraining influence exerted by the neck and labyrinthine proprioceptors upon the contralateral extensor reflex is qualitatively the same as the restraining influence exerted by the muscle's own proprioceptive inhibitors, i.e., both influences lead to increase of threshold and latency, and to diminution of size and duration of response.

SUMMARY

In the present communication is described a series of observations relating to the influence of posterior-root section and of rotation of the head upon the responses of the quadriceps muscle of decerebrate cats to contralateral and to ipsilateral break-shock stimuli applied at varying intervals (sciatic nerve). The following are the chief conclusions.

1. Under constant conditions of the neck and labyrinthine proprioceptive fields, section of the posterior root supply of the quadriceps muscle diminishes the latency and increases the rate of development, size, duration and rate of relaxation of a crossed extensor reflex set up by a single break induction shock. To use Sherrington's term, it increases the rate of "recruitment" of the individual nerve-muscle units taking part in the reflex. This is interpreted as due to the removal by posterior root section of proprioceptive inhibitors which normally are activated by sudden contraction.

2. Rotation of the head (chin toward the recording muscle) similarly diminishes the latency and increases the rate of development, size, and duration of a crossed extensor reflex set up by a single break induction shock; but it *diminishes* markedly the rate of relaxation of that reflex. This is true both before and after the posterior root supply of the quadriceps has been cut.

3. Rotation of the head (chin toward the recording muscle) causes a striking reversal in the reflex effect produced by a single break shock applied to the ipsilateral sciatic nerve. With the head normal the effect of such

a stimulus is a prompt inhibition of the "tonus" of the quadriceps muscle. With the head rotated a stimulus of the same intensity produces an equally prompt inhibition of the "tonus" of the quadriceps muscle. With the head rotated a stimulus of the same intensity produces an equally prompt augmentation of the "tonus" of the muscle. Thus, when the head is appropriately rotated, ipsilateral and contralateral stimulation both cause contraction of the extensor, but the responses from the two sides differ markedly.

4. Similar reversals occur in the response of the knee flexors, the results being in complete harmony with the principle of reciprocal innervation. With the *head normal* or with it turned away from the recording muscle, the response of semitendinosus (knee flexor) is large to ipsilateral stimulation and large to crossed, while with the *head turned* toward the recording muscle the flexor response is small or absent to ipsilateral stimulation but usually completely absent in response to contralateral stimulation.

The significance of these observations in relation to the coördination of movement is discussed.

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A MICROMETHOD FOR DETERMINATION OF THE ABSOLUTE BLOOD VOLUME, WITH DATA UPON THE BLOOD VOLUME OF THE GUINEA PIG, WHITE RAT, RABBIT AND CAT

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The method for blood volume determination herein described utilizes procedures originated by Keith, Rowntree and Geraghty (1915), and by Smith (1920). It possesses several distinct advantages over the older methods, namely: *a*, one-hundredth (0.01) of a cubic centimeter of blood suffices for a single determination; *b*, one can measure the blood volume as readily in the small laboratory animals as in the large; and *c*, owing to the small amount of blood withdrawn a number of samples may be taken simultaneously from different parts of the body and the uniformity of the dye-blood mixture ascertained.

Against these advantages, which clearly apply to but certain situations, one may place the preparation of the capillary color scales and the tiresome task of accumulating a sufficient number of capillary collecting tubes. It would seem to us improbable that the method can supplant the usual blood volume procedures in work upon normal men or upon the larger animals where a loss of 5 to 10 cc. of blood is of no consequence. But where disease has imposed circulatory abnormalities, making it difficult or impossible to judge the time needed for even reasonably uniform distribution of injected dye, and in infants and small animals where small specimens of blood must be used, the method has very obvious advantages.

TECHNIQUE. *Collecting tubes and capillary color scale.* The method employs a capillary scale (fig. 1), the tubes of which contain different known dilutions of vital red. The blood samples for actual estimation are sealed in similar capillary tubes, and, after centrifugalizing, the dye concentration in their plasma is determined by comparison with the tubes of the standard scale. The same capillary sampling tube serves also for measurement of the relative concentrations of corpuscles and plasma, i.e., is used as an hematocrit tube.

The capillary color scale is made of glass tubing, with a 1.5 mm. wall and an 8 to 10 mm. outside diameter, drawn to capillaries and cut in pieces from

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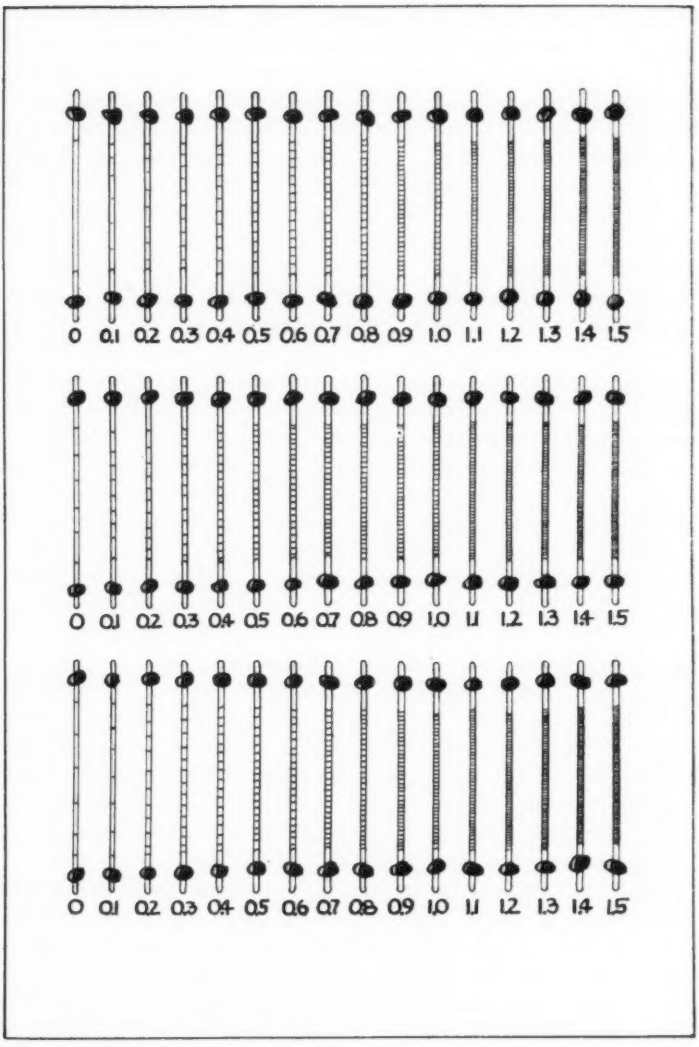

Fig. 1. Capillary colorimetric scale. Three groups of tubes on a white background.

8.5 to 9.0 cm. long. Several hundred such thin-walled capillaries are prepared. Considering that from the rat up to man the use of capillaries with an 0.5 to 0.8 mm. inside diameter is best and that on the frog and the mouse those with an 0.1 to 0.2 mm. diameter are needed, one selects those tubes with exactly the same bore. This selection is made by measuring the capillarity of the tubes in ether. After obtaining a supply of tubes of equal bore, those to be used for blood collection are given a coating of heparin. One-half per cent heparin in glass distilled water is drawn through the tubes which are then dried. The heparin used should be as nearly colorless as possible.

The dye, the concentration of which is to be determined as the final step in blood volume measurement, is diluted by plasma. In the low concentrations of vital red, with which one must work in such cases, the color of the dye does not cover perfectly the color of the plasmas. By combination of a number of plasmas with different amounts of dye, it was found that three groups of plasmas cover ordinary needs. The first representative of these three groups is cat plasma, which is almost perfectly colorless; the last one is horse plasma, which, through high lipochrome content, has a very distinctive yellow tinge. Between these two lies the color of guinea-pig plasma. A 1 per cent water solution is prepared from the dye substance and diluted by the three differently colored plasmas. Three color scales result. Each dilution is put in a capillary tube, the ends sealed, and the tube fixed upon a white surface (fig. 1). The first member of each scale (0) is the dye-free plasma with which the following dilutions were made. The second capillary tube of each scale contains a 1:1000 dilution of the 1 per cent solution, that is, 0.1 per cent volume of the same; the third, 0.2 per cent; the fourth, 0.3 per cent; the fifth, 0.4 per cent; the sixth, 0.5 per cent; the seventh, 0.6 per cent; the eighth, 0.7 per cent; the ninth, 0.8 per cent; the tenth, 0.9 per cent; the eleventh, 1.0 per cent; the twelfth, 1.1 per cent; the thirteenth, 1.2 per cent; the fourteenth, 1.3 per cent; the fifteenth, 1.4 per cent; and the sixteenth, 1.5 per cent.

Thus, the numerals below the capillaries express the per cent volume of the 1 per cent water solution. The percentage of the dye substance in samples could be calculated by dividing these numbers by 100. However, as will be seen later, the figures expressing the quantity of the 1 per cent solution make the calculation of blood volume more simple. In the event of difficulties with color matching the number of scales may be increased, using suitable colored plasmas for dilution.

Single blood volume determinations. Guinea pig: Since struggling and excitement are to be avoided in blood volume work, the animal is given 2 to 2.5 cc. of 5 per cent sodium barbital intraperitoneally. The right external jugular vein and left carotid artery are exposed, using novocaine in the skin if necessary. The right ear is shaved and coated lightly with

vaseline. Capillary tube *1* is now filled with blood taken from a small cut made in the ear and is sealed and set aside. The right external jugular vein is cannulated and 0.05 to 0.1 cc. of 1 per cent vital red per 100 grams of animal is injected. Two and one-half minutes after this injection the left carotid artery is tied and made ready for cannulation. At 3½ minutes a cannula is slipped into the incised artery but not tied in place, and the clamp released sufficiently to allow filling with blood. The clamp is then

TABLE 1

Comparative readings of same dye concentrations by five individuals using the Duboscq colorimeter and capillary scale

TEST NUMBER		DYE CONCENTRATION	DUBOSCQ	CAPILLARY SCALE
		per cent	*per cent*	*per cent*
I	1	0.003	0.0032–0.0023	0.003–0.003
	2	0.005	0.0042–0.0040	0.005–0.006
	3	0.008	0.0055–0.0058	0.008–0.009
	4	0.010	0.0066–0.0066	0.010–0.011
II	1	0.003	0.0037–0.0037	0.003–0.003
	2	0.005	0.0055–0.0055	0.005–0.005
	3	0.008	0.0077–0.0076	0.008–0.008
	4	0.010	0.0076–0.0083	0.010–0.011
III	1	0.003	0.0040–0.0040	0.003–0.003
	2	0.005	0.0053–0.0062	0.006–0.006
	3	0.008	0.0090–0.0090	0.009–0.009
	4	0.010	0.0100–0.0083	0.010–0.011
IV	1	0.003	0.0029–0.0030	0.003–0.003
	2	0.005	0.0052–0.0050	0.006–0.006
	3	0.008	0.0083–0.0071	0.009–0.009
	4	0.010	0.0083–0.0100	0.011–0.010
V	1	0.003	0.0025–0.0022	0.003–0.003
	2	0.005	0.0035–0.0037	0.006–0.006
	3	0.008	0.0058–0.0055	0.009–0.008
	4	0.010	0.0100–0.0077	0.010–0.010

closed and the cannula withdrawn. Capillary tube *2* is at once filled with this arterial blood and sealed. The mixing period in the normal guinea pig is between 3 and 3½ minutes. If the observer cares to check this point other capillary tubes may be filled with capillary blood from the ear at 2½ minutes, 3 minutes, and immediately after taking the arterial sample. These specimens will check the thoroughness of the mixing found in tube *2*. Should it be desirable to make determinations without anesthesia or in animals which are to recover, the dye may be injected into the heart or

TABLE 2

Blood volume in different animals by the capillary method

ANIMAL	WEIGHT	BLOOD VOLUME	BLOOD VOLUME IN BODY WEIGHT*	
	grams	*cc.*	*per cent*	
Guinea pig	480	32.0	6.6	
	436	23.5	5.4	
	435	28.4	6.5	
	430	30.0	6.9	
	410	23.8	5.8	
	398	28.6	7.2	
	365	24.5	6.7	
	358	23.6	6.6	
	357	20.0	5.6	
	342	21.9	6.4	
	323	20.6	6.2	
	305	20.7	6.8	
	290	20.0	6.8	
	287	18.9	6.6	
	280	16.5	5.9	
	280	18.7	6.7	
	274	16.7	6.1	
	271	17.1	6.3	
			6.4	Average
Rat	321	22.1	7.1	
	280	19.6	7.0	
	244	19.0	7.8	
	197	15.2	7.7	
	195	14.6	7.5	
	193	14.5	7.5	
	190	15.0	7.9	
	190	13.3	7.0	
	189	13.0	6.9	
			7.4	Average
Rabbit	3200	272.0	8.5	
	3050	295.8	9.7	
	3000	352.1	11.7	
	2850	248.0	8.7	
	2800	238.8	8.5	
	2650	241.1	9.1	
	2400	182.7	7.6	
	2400	211.2	8.8	
	2300	207.0	9.0	
	2300	202.4	8.8	
	2100	150.5	7.1	
	2100	168.0	8.0	
	2000	160.8	8.0	
			8.7	Average

* The gram value of the blood volume expressed in cubic centimeters can be obtained by multiplying by 1.06.

TABLE 2—*Concluded*

ANIMAL	WEIGHT	BLOOD VOLUME	BLOOD VOLUME IN BODY WEIGHT*	
	grams	*cc.*	*per cent*	
Cat	3333	210	6.3	
	3323	226	6.8	
	3193	182	5.7	
	2850	190	6.6	
	2700	144	6.0	
	2017	121	6.0	
			6.2	Average normals
	3113	137	4.4†	
	2905	122	4.2†	
	2708	130	4.8‡	

† 50 cc. of blood drawn 10 minutes before determination.
‡ 30 cc. of blood drawn 10 minutes before determination.

intravenously after the method of Rous (1918), and all blood specimens taken from the ear. Subcutaneous blood will give satisfactory results, agreeing entirely with arterial or venous blood, if the area punctured is in a state of vasodilatation and active circulation at the time of dye injection and specimen withdrawal. Blood taken from the skin capillaries during vasoconstriction, or even more markedly when the circulation is failing, will not show dye concentrations equal to those found in the large arteries unless an abnormally long period exists between dye injection and sampling, and one cannot use such long periods without entering the time when dye has begun to leave the vascular system. Blood volume determinations by a dye method may be relied upon to give a measure of the amount of blood in active circuit. In the presence of a failing circulation the active movement of blood may be confined to a relatively reduced vascular area, and specimens of blood taken from the poorly circulated skin will show different concentrations of dye than those from large vessels.

Rat and frog: The technique described for the guinea pig may be applied directly to the rat. The second specimen should be taken $4\frac{1}{2}$ minutes after dye injection, and the tail offers a convenient source of subcutaneous samples. Superficial narcotization is accomplished by 1.5 to 2.0 cc. of 5 per cent sodium barbital. Details of the use of the method for the frog will be given in a later paper dealing with these animals alone.

Calculation of the blood volume in single determinations. At the end of the experiment there are two blood samples in our possession. One was taken before the injection of the dye (sample I), the other after it (sample II). Both capillaries are now centrifugalized at 2500 revolutions for one

hour. After centrifugalization the color of the plasma of sample I is compared with that of the first (marked 0) members of the scales in order to select the correct scale for the actual colorimetry.

One reads in millimeters the length of plasma + blood cell column, v, and the length of the plasma column, p, of sample II. The color of the plasma is then compared with the standards of the corresponding scale, and thus concentration of the dye is determined, c. Besides these data the amount of the injected dye is known, q.

Hence: Plasma volume (pv) $= \frac{q \times 100}{c}$

Plasma per cent ($p\%$) $= \frac{p \times 100}{v}$

Total blood volume (tv) $= \frac{pv \times 100}{p\%}$

Blood cell volume (cv) $= tv - pv.$

Example: October 2, 1928. Guinea pig. Weight, 410 grams. 10:30 a.m.: 2.5 cc. of 5 per cent sodium barbital intraperitoneally. 11:10–11:15 a.m.: operation, cannulation of the right jugular vein and preparation of the left carotid artery. 11:18 a.m.: sample I from the right ear. 11:20 a.m.: 0.08 cc. of 1 per cent dye solution injected into the jugular vein. 11:23 a.m.: cannulation of the left carotid artery. 11:23½ a.m.: sample II from the carotid artery. 11:25 a.m.: centrifugalization begins. 12:25 p.m.: centrifugalization ends.

$q = 0.08$ cc.
$v = 62$ mm.
$p = 33.5$ mm.
$c = 0.6$ per cent

Plasma volume, $pv = \frac{0.08 \times 100}{0.6} = 13.3$ cc.

Plasma per cent, $p\% = \frac{33.5 \times 100}{62} = 54.0$ per cent

Total blood volume, $tv = \frac{13.3 \times 100}{54} = 24.6$ cc.

Cell volume, $cv = 24.6 - 13.3 = 11.3$ cc.

Repeated blood volume determinations. In observations upon the guinea pig one may use the same manipulative technique as for single determinations. If, however, the carotid artery is tied and a number of samples taken following an equal number of dye injections, it is necessary to waste the first two or three drops of blood which enter the sampling cannula, since these consist of blood which has rested in the "dead end" of the carotid artery and hence do not reflect the concentration of the circulating dye. Cutaneous blood may be used for the entire series of samples provided the observer is assured the circulation remains normal during the period of observation. If such samples are employed it is imperative that

each one be taken from a skin area where the blood flows freely and without squeezing or manipulation.

After taking the first blood sample, Ia, the first dye injection, q_1, is made. In $3\frac{1}{2}$ minutes the second sample, Ib, is secured. Before making the next dye injection, q_2, a sample of blood, IIa, is secured and, again, $3\frac{1}{2}$ minutes later, sample IIb is drawn. The same steps are followed for the third, fourth, etc., determinations.

Calculation of the blood volume on repeated determinations. Sample Ia indicates the capillary scale to be employed. Using sample Ib, the blood volume is then calculated as in single determinations. The dye concentration in sample IIa is then measured, c_{2a}, as is also the concentration in IIb, c_{2b}. The length of the plasma blood cell column, v_2, and the plasma column, p_2, are measured in millimeters and the calculation of the second blood volume, tv_2 is as follows:

$$pv_2 = \frac{q_2 \times 100}{c_{2b} - c_{2a}}$$

$$p\%_2 = \frac{p_2 \times 100}{v_2}$$

$$tv_2 = \frac{pv_2 \times 100}{p\%_2}$$

$$cv_2 = tv_2 - pv_2$$

Subsequent determinations may be made in the same manner.

Example: October 9, 1928. Guinea pig. Weight, 395 grams. 2:10 p.m.: 2.0 cc. of 5 per cent sodium barbital intraperitoneally. 3:00 p.m.: operation, cannulation of the left jugular vein. 3:02 p.m.: blood sample Ia. 3:05 p.m.: 0.07 cc. of 1 per cent dye solution intrajugularly. 3:08½ p.m.: blood sample Ib. 3:35 p.m.: blood sample IIa. 3:37 p.m.: 0.07 cc. of 1 per cent dye injected. 3:40½ p.m.: blood sample IIb. 4:05 p.m.: blood sample IIIa. 4:07 p.m.: 0.07 cc. of dye. 4:10½ p.m.: blood sample IIIb. Centrifugalization.

$q_1 = 0.07$ cc. $\quad pv_1 = \frac{0.07 \times 100}{0.5} = 14$ cc.

$v_1 = 57$ mm. $\quad p\%_1 = \frac{31 \times 100}{57} = 54.5$ per cent

$p_1 = 31$ mm. $\quad tv_1 = \frac{14 \times 100}{54.4} = 25.7$ cc.

$c_1 = 0.5$ per cent $\quad cv_1 = 25.7 - 14 = 11.7$ cc.

$q_2 = 0.07$ cc. $\quad pv_2 = \frac{0.07 \times 100}{0.85 - 0.3} = 12.7$ cc.

$v_2 = 48$ mm. $\quad p\%_2 = \frac{24.5 \times 100}{48} = 51$ per cent

$p_2 = 24.5$ mm.

c_{2a} = 0.3 per cent $\quad tv_2 = \dfrac{12.7 \times 100}{51} = 24.9$ cc.

c_{2b} = 0.85 per cent $\quad cv_2 = 24.9 - 12.7 = 12.2$ cc.

q_3 = 0.07 cc. $\quad pv_3 = \dfrac{0.07 \times 100}{1.05 - 0.55} = 14$ cc.

v_3 = 61 mm.

p_3 = 33 mm. $\quad p\%_3 = \dfrac{33 \times 100}{61} = 54.1$ per cent

c_{3a} = 0.55 per cent $\quad tv_3 = \dfrac{14 \times 100}{54.1} = 25.8$ cc.

c_{3b} = 1.05 per cent $\quad cv_3 = 25.8 - 14 = 11.8$ cc.

CONTROL TESTS WITH THE DUBOSCQ COLORIMETER. A series of known solutions was made up in heparinized plasma. Different members of the department, of varied experience in colorimetry, then read the dye concentrations by means of the Duboscq colorimeter and the capillary scale. The results are given in table 1 and are clearly better for the capillary scale than for the Duboscq. This fact must impress all who make such readings. When vital red is diluted with plasma a color results which at best is not read with assurance upon the colorimeter, and if the plasma is at all turbid or shows traces of hemolysis the reading becomes a matter of judgment merging rapidly into guess-work. Lindhard (1926), in a paper dealing with the errors in dye methods for blood volume work, compared a procedure utilizing tubes with an outer diameter of 6 mm. and length of 6 cm. as containers for 1 cc. of specimen and standard with the results obtained by means of Bürker's colorimeter. He obtained more satisfactory results with the colorimeter. Our experience has been with tubes of much smaller bore and with a somewhat different colorimeter. For routine work in which plasmas of many different types may be met, the capillary-tube method has impressed us as being certainly as exact as the colorimeter technique and possibly decidedly better, but, as was pointed out in the beginning of the paper, the real advantages of the new method rest upon physiological rather than upon technical grounds.

BLOOD VOLUME IN CERTAIN LABORATORY ANIMALS. In table 2 a series of measurements of blood volume is given for some of the smaller laboratory animals.

The figures for the guinea pig cannot be compared with other results by dye methods. Erlanger (1921) presents a few figures for these animals obtained by extraction methods and falling between 3.5 and 5.5 per cent of body weight. These results give the guinea pig a uniquely low blood volume as compared with other mammals that have been studied. Nine determinations by Dreyer and Ray (1910–11) included in Erlanger's (1921) summary fell between 5.17 and 3.32 per cent of body weight.

In the case of the rat, Chisholm (1911) found blood volumes ranging between 5.6 and 7.4 per cent of body weight. He employed a washout and extraction method. Cartland and Koch (1928), using a dye method, obtained an average value of 6.8 per cent in a series of eight animals. Our figure is 7.4 per cent in a series of nine animals.

For the rabbit, the results reported by us are uniformly high and the average 8.7 per cent of body weight is more in the range of values reported for the dog. The figure 6.2 per cent of body weight obtained for the cat is practically identical with figures gained by a variety of methods (Erlanger, 1921).

Discussion. The method of Cartland and Koch (1928) to which reference has been made is also called a micromethod and uses vital red. One-fourth to one-half a cubic centimeter of blood is required against one-hundredth of a cubic centimeter or less in the procedure we have described. The withdrawal of 0.5 cc. of blood—and with such specimens Cartland and Koch have obtained impressively uniform results—is not permissible as a repetitive manoeuvre in animals even of the size of rats, and a single withdrawal of this magnitude cannot be considered in the frog where the blood volume in animals of ordinary size ranges between 2.0 and 2.5 cc.

SUMMARY

1. A method for determining blood volume after injection of vital red is described.

2. This method utilizes heparinized blood collected in capillary tubes in amounts not greater than 0.01 cc.

3. After centrifugalization the relative cell and plasma volumes in these tubes are measured and the dye concentration in the plasma read against a previously prepared series of tubes containing known mixtures of dye in plasma.

4. The small amount of blood withdrawn enables one to measure blood volume in animals of the size of frogs and renders it possible to make repeated determinations of blood volume in small animals.

5. It is also possible to withdraw many specimens from different parts of the body and so determine the distribution of the dye and the true "mixing time."

The use of capillary tubes for colorimetric work was suggested to us by Dr. A. N. Richards in connection with a necessity to determine the blood volume of frogs. It is a pleasure to acknowledge our indebtedness to him and our appreciation of his permission to publish the method as applied to blood volume measurement prior to his own publication in connection with micro-quantitations in kidney work.

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BLOOD VOLUME IN THE GUINEA PIG DURING ANAPHYLACTIC SHOCK

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Simonds (1925) showed that the blood volume of "most" dogs is reduced during anaphylactic shock. This is in accord with physiological expectations, since in these animals there can be no doubt that the essential gross phenomenon of anaphylaxis is obstruction to the flow of portal blood through the liver. As a result of such an obstruction fluid leaves the very permeable liver capillaries and to a less degree the capillaries throughout the intestinal tract. The reaction rests upon a simple mechanical basis and merely duplicates the classical lymph flow experiment of obstructing the inferior vena cava just above the diaphragm.

Owing to the fact that the acute anaphylactic seizure has been associated with a possible liberation of histamine into the circulation and thus with capillary dilatation and increased vascular permeability, it has seemed worth while to make a small series of blood volume measurements during anaphylactic shock in the guinea pig. These animals are entirely free from the obstruction to the portal circulation which occurs in the dog. The single specific circulatory reaction shown by the guinea pig is a fall in pulmonary arterial pressure (Went and Drinker, 1929a), and there is consequently no special reason for change in blood volume such as one sees in the dog. If, therefore, blood volume altered during anaphylactic shock, the change would indicate a general circulatory effect expressed widely through the capillaries rather than a simple obstructive phenomenon akin to that noted in the dog.

EXPERIMENTS. Guinea pigs were sensitized with 2 cc. of sheep serum given intraperitoneally, and were used for experiments during the third week following the sensitizing injection.

The onset of anaphylaxis was recorded as described in an earlier paper (Went and Drinker, 1929a). Blood volume was determined repeatedly by the micromethod of Went and Drinker (1929b).

A single protocol will indicate the course of a typical experiment.

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October 24, 1928. Sensitized guinea pig. Weight, 398 grams.

1:30 p.m. 4 cc. of 5 per cent sodium barbital intraperitoneally.
2:40 p.m. Cannulation of right external jugular vein and exposure of left carotid artery.
3:01 p.m. 0.5 cc. of 1 per cent curare injected intravenously. Artificial respiration.
3:04 p.m. Vital red injection.
3:08 p.m. Removal of sample I from the carotid artery for blood volume determination. Result: blood volume = 28.6 cc.
3:10 p.m. 0.5 cc. sheep serum injected intravenously.
3:10:30 p.m. Air begins to be excluded from the lungs, indicating the onset of the bronchoconstriction characteristic of anaphylactic shock in the guinea pig.
3:10:50 p.m. Air overflow closed so that asphyxia was checked.
3:11:30 p.m. Removal of sample II from the carotid artery for determination of dye concentration.
3:11:40 p.m. Increased artificial respiration to prevent asphyxia.
3:14:20 p.m. Vital red injection.
3:18:30 p.m. Removal of sample III from carotid artery for blood volume determination. Result: blood volume = 28.1 cc.
3:20:40 p.m. Vital red injection.
3:24:50 p.m. Removal of sample IV from carotid artery for blood volume determination. Result: blood volume = 28.6 cc.
3:26 p.m. Animal killed. At autopsy the lungs collapsed. The experiment was an instance of mild anaphylactic shock and was carried through without asphyxia.

Discussion. In our experience, if asphyxia was prevented no change occurred in blood volume. When shock was exceedingly severe and some degree of asphyxia unavoidable, the blood volume reading during the height of the asphyxia was low due to poor mixing of the injected dye. If such animals recovered through adjustment of artificial respiration a subsequent blood volume determination showed a figure very close to that obtained before shock.

SUMMARY

Anaphylactic shock was produced in seven guinea pigs sensitized with sheep serum. The blood volume was measured by the micromethod of Went and Drinker (1929b), and the onset of shock determined by the overflow procedure described by the same authors (Went and Drinker, 1929a). No changes in blood volume were found when the complicating factor of asphyxia was prevented by careful adjustment of artificial respiration.

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I. RESPIRATORY RESPONSES TO ACOUSTIC STIMULATION IN INTACT AND DECEREBRATE ANIMALS[1]

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In general, the effect of sound on an organism is regarded as being in some way associated with psychologic or instinctive reactions. The exhilarating or depressing influence of music on normal or abnormal persons, or on the higher mammals, such as dogs, is usually thought of as a purely emotional effect. A sudden, unexpected sound causes an unquestionable physical upset, but this effect is again regarded as an instinctive reaction to the emotion of fear. According to the now generally accepted James-Lange (1922) theory of the emotions, however, the mental component of any reaction, the thing felt, the emotion itself, is secondary and entirely dependent on the primary physical disturbance. Such being the case, it is logical to view the physiologic responses to external stimuli as reflexes, and it has been our purpose to investigate the visceral reactions to acoustic stimuli in this light.

To do this it became necessary to bring under control both the physical and the physiologic factors. It has been the aim, on the one hand, to use only acoustic stimuli whose frequency, wave form, duration and intensity were known quantities and, on the other hand, to eliminate or at least to minimize psychic as well as extraneous somatic impressions in the experimental animals. An electric oscillator of the Cambridge type has been found very satisfactory as a means of actuating a loud speaker to produce the stimuli. With this instrument, acoustic stimuli of known wave form, duration and frequency can be obtained in variable and repeatable intensities. These stimuli are pure tones up to a frequency of about 4000. From 4000 to 8000, the upper frequency limit, the tones become complex due to the presence of harmonics. Occasionally other complex sounds were used as the stimuli for comparison with the oscillator tones.

The physiologic factors were more difficult to control. In order to minimize extraneous somatic impulses, the animals were made comfortable in

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a darkened room and kept in as normal a position as possible during the experiments. To exclude the psychic factor, or at least to reduce it, several methods were employed: training, repetition, sleep and decerebration. Dogs were taught to remain quietly on the table for a number of days prior to the experiments. They were permitted to hear the same stimuli as were used for the tests during these preliminary periods of training, so that when the actual experiments were performed there was never any evidence of emotional response. Because of the difficulty of training cats, a different procedure was adopted. The necessary apparatus was first adjusted, then the animals were put in a comfortable cage, and given time to curl up and doze before any tests were made. Frogs and rabbits were used repeatedly for the same type of experiment, sometimes every day successively for several weeks, so that any effect which might be observed could not ultimately be said to be the result of an entirely new environmental factor. The most radical method of excluding cortical influences is, of course, decerebration. This was done with frogs and rabbits. The frogs were anesthetized with urethane and the cerebrum removed above the optic lobes. The rabbits were decerebrated above the thalami by the method used by Magnus under ether anesthesia.

The investigations include, so far, observations of changes in respiratory and cardiac rhythm, in blood pressure, and in splenic and renal volumes. The present report is an account of the respiratory responses to acoustic stimulation occurring in normal and decerebrate animals. The results of the other experiments will be reported in subsequent papers.

EXPERIMENTAL RESULTS. *Frogs.* In this series of experiments ninety-one observations were made on five intact bullfrogs (*Rana catesbiana*).

The arrangement of the apparatus for the experiments was very simple. The frog was placed in a shallow dish on a tripod. A thin wooden lever was adjusted so that one end rested lightly under the frog's throat. The movements imparted to it by the respirations were recorded on the revolving drum of a kymograph. A small loud speaker connected with the oscillator was placed about 30 cm. behind the animal. Everything but the kymograph was enclosed in a cardboard box in order to exclude light. A narrow vertical slit was made in the box for the writing end of the lever.

The stimuli used were the oscillator tones or the sound of the bell of an alarm clock. The oscillator tones were exhibited either as prolonged sounds or as short repeated sounds lasting less than a second.

The results of the experiments were uniform and definite. Prolonged stimulus caused decrease in the respiratory rate and decrease in the amplitude of respiration, whereas short repeated stimuli or the sound of the alarm clock bell caused an increase in rate without marked change in amplitude. Figure 1 represents a typical respiratory response of a bullfrog to acoustic stimulation. The stimulus was a sound having a frequency of

512 each second with a duration of twelve minutes. The respiratory rate, between 44 and 48 each minute, decreased to between 40 and 42 each minute during the period of stimulation. After the cessation of the stimulus, the original rate was resumed.

Before any of the curves presented in this paper were plotted, kymographic records indicated that the rate of respiration had been fairly constant over a sufficient period before the application of the stimulation. Prolonged prestimulation curves have been omitted. Figure 2 shows the respiratory change occurring during a short period of stimulation with the bell of an alarm clock. A rapid increase in rate accompanies this type of stimulus. At the end of the period of acoustic stimulation, the rate decreases to the original rate. The same type of response as shown in this curve also occurs when the stimulus is a series of short, repeated oscillator tones.

After the respirations of the intact frogs had been studied, two of the animals were decerebrated, under urethane anesthesia, above the optic lobes, and twenty-nine observations were then made. The same general results were noted. During prolonged stimuli the rate decreased; during short, repeated stimuli, the rate increased. The sounding of the bell also was accompanied by an increase in rate. Figure 3 shows the respiratory response of a decerebrate frog to a stimulus consisting of an oscillator tone of 512 vibrations each second lasting four minutes. The respiratory rate had varied between 57 and 65 before stimulation. During the period of stimulation it dropped to 53. After the stimulus there was again an increase in the rate. The respiratory response in a decerebrate frog to a series of short sounds is shown in figure 4. The frequency of the stimulus was 2048. The rate each second (52 to 53 before the stimulus) went up to 58.5 during the period of stimulus. The rate went down to the original range after the cessation of the sounds.

The complete range of frequency of the stimuli in these experiments was from 128 to 8000 vibrations each second. A study of the records showed that the pitch of the stimulus was not a factor in determining the extent or the direction of the response. These responses were conditioned by the type of stimulation: short, repeated sound stimuli or bell sounds causing an effect which was more rapid, more marked and opposite in direction to that accompanying prolonged sounds.

Mammals. Two each of intact rabbits, cats and dogs were used and a total of sixty-four experiments was made. Precautions were taken to eliminate extraneous disturbances as much as possible and to avoid emotional reactions.

The tracings were obtained by using a pneumograph made of a piece of corrugated rubber tubing about 15 cm. long such as is used on standard apparatus employed in metabolism. One end of the tube was closed with

a rubber stopper, the other with a rubber stopper through which a glass tube had been inserted. This glass tube was connected with a Marey tambour whose writing lever recorded the respiratory movements. This pneumograph was found very satisfactory since it was soft and pliable and at the same time very sensitive and could be lightly tied around the thorax of the animal with a gauze bandage without causing any irritation.

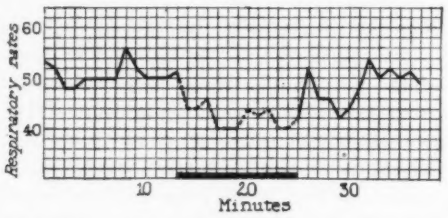

Fig. 1. Respiratory changes in a frog during a prolonged acoustic stimulus (512 vibrations each second). In this figure, and in figures 2, 3 and 4, the abscissas represent the actual number of respirations each minute and the ordinates the successive minutes of that part of the record shown in the curve. The period of stimulation is indicated both by the heavy horizontal line and by the dotted portion of the curve.

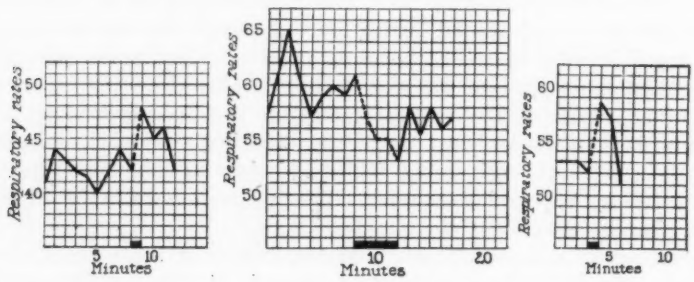

Fig. 2 Fig. 3 Fig. 4

Fig. 2. Respiratory changes in an intact frog during stimulation with the sound of an alarm clock bell.

Fig. 3. Respiratory changes in a decerebrate frog during stimulation with a prolonged acoustic stimulation (512 vibrations each second).

Fig. 4. Respiratory change in a decerebrate frog during stimulation with a series of short sounds (2048 vibrations each second).

In studying the records of the respirations it was found that many of the changes were sudden or transient. These effects would have been obscured entirely if group counts, representing rates alone, had been made. For this reason each respiration was measured and its duration estimated to twentieths of a second.

The stimuli used were oscillator tones of 256, 2048 and 6500 vibrations

each second. These stimuli were exhibited as single prolonged sounds, repeated prolonged sounds, single short sounds or series of repeated short sounds. They were conveyed through a loud speaker at a distance of 1 meter from the animal's head.

Four effects were noted in each experiment: 1, the immediate effect of onset of the stimulus; 2, the effect during the period of stimulation; 3, the immediate effect of cessation of the stimulus, and 4, the after-effect.

The immediate effect on respiration of an acoustic stimulus was variable. It was present in only 25 to 30 per cent of the experiments and took the

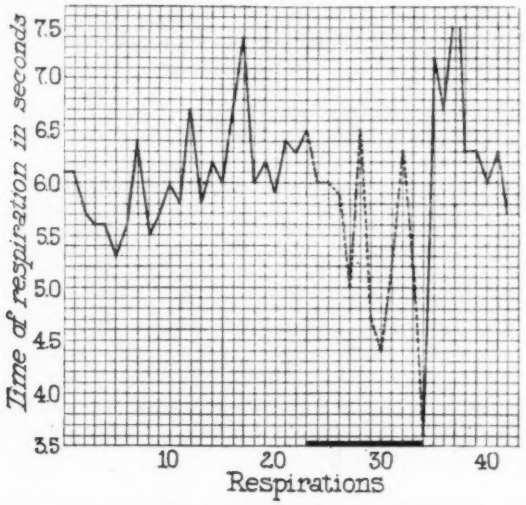

Fig. 5. Respiratory changes in a dog during and following an acoustic stimulus having a frequency of 256 each second and lasting one minute. In this and subsequent respiratory curves, the ordinates represent the time in seconds of the individual respirations and the abscissas represent the successive respirations. The period of stimulation is indicated both by the horizontal black line and by the dotted portion of the curve.

form either of an increase or of a decrease of the time of the respiration then in progress.

During the period of stimulation the effect was much more constant. The usual change was a decrease in the length of the respirations, whether the stimulus consisted of a prolonged sound or of a series of repeated sounds. This effect differed from that observed in the preceding group of experiments in that the respirations of the frog decreased in length only during a series of repeated stimuli, whereas they increased during a prolonged period of stimulation.

The effect of the cessation of the stimulus was again variable. It was positive in 25 to 30 per cent of the observations and, as in the case of the immediate effect of the onset of the stimulus, consisted either of an increase or of a decrease in the time of respiration then in progress.

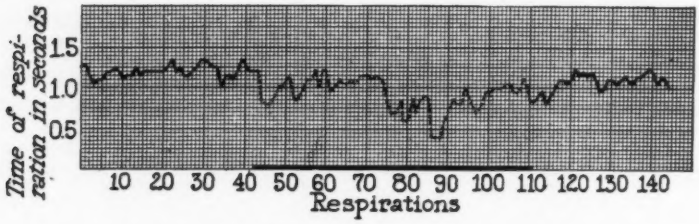

Fig. 6. Respiratory changes in an intact rabbit during and following an acoustic stimulus of 2048 vibrations each second and lasting one and a half minutes.

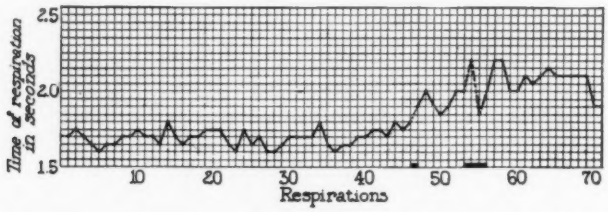

Fig. 7. Respiratory changes in a decerebrate rabbit during and following two separate acoustic stimuli at 2048 vibrations each second, lasting six-hundredths of a second and six seconds respectively.

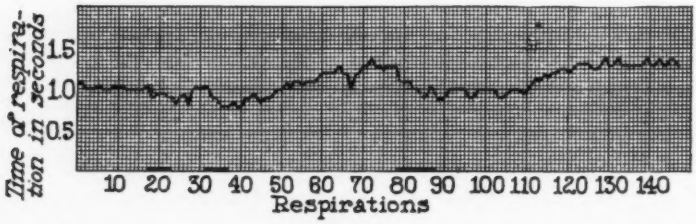

Fig. 8. Respiratory changes in a decerebrate rabbit during and following three separate acoustic stimuli at 2048 vibrations each second, lasting seven, six and ten seconds respectively.

The after-effect was constant. When the stimulus had ceased, the respirations increased in length, often to considerably above their duration before stimulation. Figure 5 represents a typical respiratory curve in

a dog during an acoustic stimulation of 256 vibrations each second and lasting one minute. All four effects are demonstrated here. The immediate effect was a shortening of the time of the first respiration during the period of stimulation. During the progress of the stimulus the time of respirations decreased. At the close of the stimulus, there was a marked increase in the time of respiration then in progress. This increase persisted as an after-effect for several respirations before the original rate of respiration was resumed. In figure 6 may be seen the respiratory curve of a rabbit during stimulation with a sound of 2048 vibrations each second and lasting one and a half minutes. The immediate effect of the stimulus was again a shortening of the time of respiration in progress. There was a general decrease in the time of respiration during the period of stimulation, no immediate change on cessation of the stimulus, and a gradual return to the original range as an after-effect. The persistent lengthening of the time of the respirations to values far above their original range after the cessation of the stimulus (as shown in fig. 5) is a variable phenomenon. A change in the direction of an increase in time of respiration takes place almost without exception, whether the original range is surpassed for a time or merely resumed.

A series of experiments was carried out on rabbits which had been decerebrated above the thalami by the method employed by Magnus under ether anesthesia. A total of seventy tests was made on fifteen animals. The respiratory changes due to acoustic stimulation were exactly the same in these animals as those noted in the intact animals. That is to say, there was a variable immediate effect, a decrease in the time of the respirations during the period of stimulation, a variable effect with the cessation of the stimulus and an increase in the time of respiration after the stimulus. Figure 7 represents a portion of a respiratory record of a decerebrate rabbit showing the effects of two acoustic stimuli (both having a frequency of 2048 each second), the first period of stimulation lasting six-hundredths of a second and the second six seconds. The first stimulus caused an immediate increase in the time of the respiration in progress. After the cessation of the stimulus there was a persistent increase in the time of the respirations. The second stimulus was accompanied by a still further immediate increase in the time of each respiration followed by a sudden drop during its progress. At the close of the period of stimulation there was again a rise, after which there was no tendency to return to the original range until after the thirteenth post-stimulation respiration. Figure 8 gives a record of the respirations of a decerebrate rabbit during acoustic stimulation. Three stimuli of 2048 vibrations each second were used lasting seven, six and ten seconds respectively. During each period of stimulation there was a marked decrease in the time of respiration and in each post-stimulation period there was an increase.

COMMENT. The data furnished by the preceding experiments seem to show conclusively that there exists in frogs and certain mammals, such as rabbits, cats and dogs, a definite respiratory response to stimulation of the organism with acoustic vibrations. This reaction has been shown to be constant in direction in mammals and variable in direction in frogs, according to the type of stimulation. It has also been demonstrated that this response is in the nature of a reflex and not dependent on higher nervous centers, because ablation of the hemispheres does not alter either the direction or the extent of response to acoustic stimulation.

The bulbar and midbrain relations of the eighth nerve are established early in vertebrate phylogeny. Even in such primitive forms as the Cyclostomas, the dorsal branch of this nerve, representing the cochlear branch in higher forms, has all the elements of the lower connections in the mammals. It sends root fibers to its primary nuclei, and even at this stage of development a few fibers lose themselves in the diffuse gray substance of the medulla. Furthermore, there is a definite root fiber connection with the nucleus reticularis superior, a group of reticular cells lying just above the level of its entrance in the bulb. Secondary fibers then make up the ascending lateral lemniscus to the mesencephalic nuclei, and from these structures, a descending reflex tract, the tectobulbar tract, returns to the medulla and cord. This tract also gives off collaterals to the reticular formation (Kappers, 1920).

No matter how complex the connections of this nerve with higher nervous centers may have become in the higher vertebrates, its relations to bulbar and midbrain structures, which have been established in the most primitive forms, always remain patent and are probably the means of a large number of reflex visceral responses of the organism to acoustic stimuli. The fact that removal of the cerebral hemispheres does not in any way affect the respiratory responses of the animals studied in these experiments emphasizes these relations. The reactions of decerebrate frogs are not as significant as the reactions of the decerebrate rabbits, since in the amphibians there is not as yet a projection pathway for acoustic impulses to the cerebrum. The auditory connections go only as far as the midbrain, so that the decerebrate responses merely confirm the anatomic evidence of the central extent of this nerve system. In all of the mammals, however, the cerebral projection is very important, but its removal leaves intact the lower respiratory reflex pathway.

We are not, as yet, offering an explanation concerning the difference in effect of acoustic stimulation on respiration in amphibians and mammals. Although the respiratory center has not been located exactly in the amphibians, it may be considered to consist of a more or less interrupted column of cells in the dorsolateral portion of the medulla, from the posterior border of the cerebellum to the calamus scriptorius. This region

includes the nuclei of the motor nerves of respiration, the fifth, seventh, ninth and tenth nerves, along with certain reticular cell groups. The connection between the auditory nerve and this "center" may be effected through direct root fibers, through reticular cells associated with the lateral lemniscus, or through collaterals of the descending tectobulbar tract.

The reason for a difference in the direction of the effects, dependent on whether a prolonged or a repeated stimulus was used, will probably appear when more is known concerning the electric phenomena in the auditory nerve accompanying acoustic stimulation.

The quadrigeminate bodies are known to exert a significant influence on respiratory rhythm (Winterstein, 1921). The part played by this portion of the brain-stem in the acoustic responses which have been presented has not been determined. This problem is reserved for further investigations. Allen has shown that the fibers arising in the midbrain and which influence respiration in mammals do not pass directly to the cord but relay their impulses to reticulospinal cells in the medulla. There exists, however, the possibility of a direct root connection, or at least a secondary auditory connection with the reticular formation at the level of entrance of the eighth nerve. The demonstration by Lumsden of an "apneustic" or inspiratory center at this level in cats is significant. The fact that there is an increase in rate of respiration with stimulation of the eighth nerve emphasizes the possibility that the reflex which we have studied may pass over more direct paths, at least in part, than those which involved the corpora postica and the descending collateral nerves.

SUMMARY

A series of investigations has been made of the visceral reactions to acoustic stimuli and these responses have been studied as simple reflexes. By various methods, extraneous somatic impressions as well as cerebral influences have been excluded or at least minimized in the experimental animals. The stimuli were furnished by means of an electric oscillator. This instrument furnishes acoustic stimuli of known wave form, frequency and duration, in variable and repeatable intensities.

In this paper, the first of a series in which various acoustic-visceral reflexes are presented, is an account of the respiratory responses to acoustic stimulation in frogs and in certain mammals. The results of the experiments in which intact animals were used were compared with those of similar experiments on decerebrate animals.

In frogs, prolonged oscillator tones caused a decrease in the respiratory rate, while short, repeated stimuli or the sound of an alarm clock bell caused an increase in rate. Decerebrate frogs gave the same reactions as intact frogs.

In the mammalian forms (rabbits, cats and dogs) studied, acoustic stimu-

lation was accompanied by an increase in the respiratory rate, a shortening of the individual respirations, whether the stimulus was a prolonged sound or a series of short sounds. After the period of stimulation, the respirations again increased in length. The immediate effect of beginning or of ending the stimuli was positive in only 25 to 30 per cent of the experiments and then consisted either of an increase or of a decrease of the time of the respiration in progress. A large number of tests was made on decerebrate rabbits and again the respiratory reflex in these animals was the same in direction and extent as in the intact animals.

The relations of the auditory nerve to structures in the medulla and midbrain are discussed from a phylogenetic standpoint. The connections of this nerve with the lower centers, established in the most primitive vertebrate forms, continue to exist in the higher forms, below the level of the later evolved projection pathways and make possible a number of visceral reflexes which do not involve the higher centers.

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II. CARDIAC RESPONSES TO ACOUSTIC STIMULATION IN INTACT AND DECEREBRATE RABBITS[1]

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This series of investigations of acoustic visceral responses involved a study of the effect of auditory vibrations on the heart rate. Rabbits were used in the experiments. The records were made with an Einthoven galvanometer. Lead II was used in all of the experiments. The hair was removed from the lower half of the right front and the left hind legs of the animal by means of a solution of sodium sulphide. The legs were then wrapped in gauze soaked with a saturated solution of sodium chloride. Copper wire wound around the gauze served as the electrodes which were led off directly to the galvanometer.

The animal was placed in a suitable box, large enough to assure comfort but small enough to prevent any turning. The box was put in a position such that its elevation brought the animal on a level with the horn of the loud speaker which transmitted the acoustic stimuli from a distance of 80 cm. A black cloth was loosely thrown over the box and the room darkened. At least ten minutes elapsed before records were taken. These conditions were repeated in each experiment.

EXPERIMENTAL DATA. Ninety-three observations were made on twenty-one intact animals. The stimuli were oscillator tones of 256, 2048 and 6500 vibrations each second, exhibited as prolonged sounds, single or repeated, or as short sounds, single or in series. When the records were developed the heart beats were measured to hundredths of a second.

The general result observed was a slowing of the heart rate during the periods of acoustic stimulation. When the stimulus was a single, prolonged sound, this effect was most marked at the beginning of the period of stimulation and tended to subside during the remainder of the period. With the release of the stimulus there often appeared a second slowing of the rate for several beats. When the stimulus was a series of repeated short sounds, on the other hand, the slowing continued uniformly throughout the period of stimulation. These effects were constant in the intact animals.

[1] Work done in the Section on Biophysical Research and the Division of Experimental Surgery and Pathology.

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Figure 1 shows a typical example of the changes in the time of the heart beats of an intact rabbit during acoustic stimulation. An immediate increase in the time of duration of the heart beats may be noted at the onset of the first stimulus. The time of the heart beat decreases during the period of stimulation but does not reach its original value. At the cessation of acoustic stimulation there is a slight secondary rise in the time of the beat, after which the value again drops. With the second stimulus the effect is similar but not so marked.

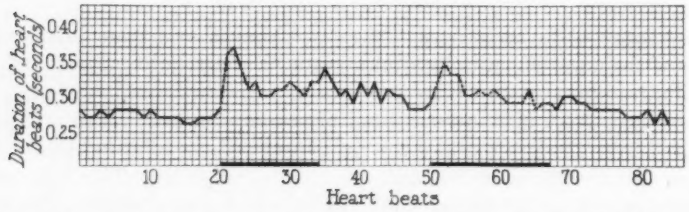

Fig. 1. Changes in the duration of the cardiac beats in an intact rabbit during two acoustic stimuli, with a frequency of 2048 vibrations each second lasting ten seconds each with an interval of ten seconds. In this and subsequent curves representing the time of the cardiac beats, the ordinates represent the time in seconds of the individual beats, while the abscissas represent the successive beats. The period of stimulation is represented by the horizontal black line and by the dotted portion of the curve.

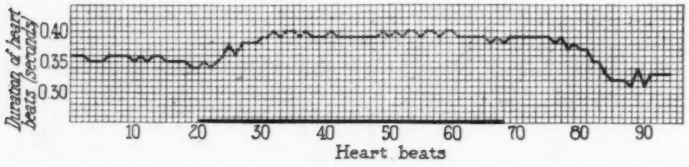

Fig. 2. Changes in cardiac rate in an intact rabbit during stimulation by a series of rapidly repeated sounds having a frequency of 6500 vibrations each second.

The change in the time of the heart beats of an intact rabbit during a series of rapidly repeated sounds is shown in figure 2. The stimuli in this experiment had a frequency of 6500 vibrations each second and lasted fifteen seconds. The time of heart beat increases with the onset of the sounds and remains elevated throughout the period of stimulation. After the cessation of the stimuli, the duration of the heart beats returns to the original range.

A series of thirty-six observations was made on fifteen decerebrate rabbits. The results in this group of experiments were quite different from those in the preceding group. The acoustic stimuli did not change the

heart beats in 60 per cent of the observations. In the remaining experiments, the effect was opposite in direction to that observed in the intact animals, for the heart beats were decreased in length. This effect was sometimes secondary to an initial increase of time or irregularity of the beats.

Figure 3 is an example of one of the positive responses of the heart of a decerebrate rabbit to a sound stimulus. The frequency of the stimulus was in this instance 6500 vibrations each second. Here there was a transient increase of duration of the beats before the decrease. This type of response was never noted in the intact animals. The time of heart beats slowly returns to its original range after the cessation of the stimulus.

COMMENT. The interpretation of the discrepancy between the cardiac responses in the intact and decerebrate animals cannot be attempted fully. A number of physiologic factors are probably involved, such as change in

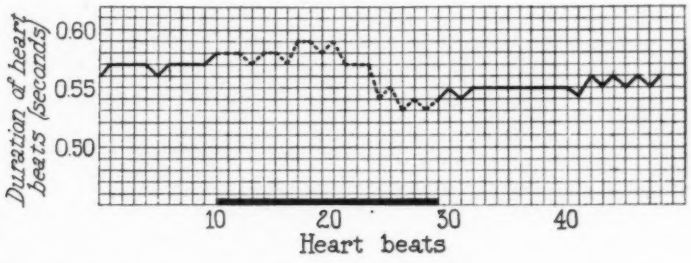

Fig. 3. Changes in the time of duration of the cardiac beats in a decerebrate rabbit during stimulation with a sound of 6500 vibrations each second.

blood pressure and body temperature, in the decerebrate preparations. Considering the nervous system alone two possibilities may be suggested: either that the cerebral arcs are involved in the reaction, or that the nervous mechanism governing the heart rate is more labile to the shock of decerebration than the respiratory centers. The same decerebrate animals were used for these experiments, as were used for studying the respiratory reflexes. All of the observations were made shortly after operation, the longest survival in this group having been nine days. It is our purpose in further investigations to examine this reflex in animals which have more fully recovered from the effects of shock. Golz, however, noted that in his decerebrate dog, which lived eighteen months, cardiac rate was not changed, even with marked pseudo-affective reactions to external stimulation, involving respiratory disturbances. This fact points to a cerebral participation in the reaction rather than that absence or reversal of response is due to a localized shock effect.

Although there is no direct connection between the dorsal motor nucleus of the vagus and the auditory fibers, still the intimate relation of both of these structures to the reticular formation would lead one to believe that a direct reflex might be possible without involvement of the higher centers. That some such path does exist is shown by the fact that in 40 per cent of the experiments on the decerebrate animals, the acoustic stimuli caused an alteration of cardiac rhythm, but one which was opposite in direction to that occurring in the intact animals. It is quite possible, then, that the response has become modified to the point of complete reversal by the cerebral arcs.

SUMMARY

Changes in cardiac rhythm brought about by acoustic stimulation were studied in a series of intact and decerebrate rabbits. The records were made by means of an Einthoven galvanometer. In the intact animals the heart rate decreased in response to the acoustic stimuli. This decrease persisted throughout the period of stimulation if a series of repeated short sounds was used, but disappeared during the course of the stimulus if a prolonged sound was used. Often a second decrease occurred at the cessation of the stimulus.

In the decerebrate animals the effect was absent in 60 per cent of the experiments, and had the opposite direction in the remainder.

Two possible interpretations are suggested for the discrepancy between the effects in the normal and decerebrate animals: either that the reactions are modified by the participation of the cerebral arcs, or that the centers governing cardiac rhythm are more labile to shock than the respiratory centers. The same decerebrate animals were used in these experiments as in those on the respiratory reflex.

III. THE EFFECT OF ACOUSTIC STIMULATION ON THE BLOOD PRESSURE OF URETHANIZED DOGS[1]

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A short series of experiments was carried out on urethanized dogs to note the effect of acoustic stimulation on the general blood pressure. Although the bulbar centers are probably little affected by urethane, nevertheless the results of these experiments are presented merely to suggest the general nature of the acoustic vasomotor reflex. The standard method for obtaining blood pressure was used, the cannula in the carotid being connected with a recording mercury manometer.

The tones of a loud speaker connected with an electric oscillator were used as the stimuli, the usual frequencies being 256, 512, 1024, 2048, 6500 and 8000 cycles each second. The loud speaker was placed 30 cm. behind the animal's head.

Three types of effects were noted: 1, a fall in pressure during the period of stimulation; 2, a rise in pressure during the period of stimulation, and 3, release effects or changes occurring at the cessation of the stimuli. Variations did not occur with all of the stimuli, but invariably accompanied the initial stimulus, a stimulus exhibited after a period of rest, or a stimulus in which a change of pitch had been introduced.

Figures 1 and 2 show rises in the blood pressure associated with acoustic stimuli. In the first tracing the stimulus was a short sound of two seconds' duration at 1024 vibrations each second. A rise in pressure of 7 mm. of mercury followed the stimulus. The second tracing shows a rise in pressure of 6 mm. of mercury during a series of short stimuli having a frequency of 360 vibrations each second.

In figures 3 and 4 a fall in pressure occurred with acoustic stimulation. In figure 3 the stimulus was a sound of 8000 vibrations each second lasting five minutes. A drop in pressure of 6.5 mm. of mercury occurred, reaching its maximum one minute and thirty seconds after the onset of the stimulus. At the cessation of stimulation there was slight irregularity before the pressure returned to normal.

[1] Work done in the Section on Biophysical Research and the Division of Experimental Surgery and Pathology.

[2] Fellow in Physiology.

Figure 4 shows the definite effect of change of pitch. Previous to the results shown in figure 4, the frequency of 256 each second had been used without producing any effect. Then the pitch was changed to 1000 vibrations each second, whereupon a sudden drop in pressure of 5 mm. of mercury occurred with immediate return to normal.

Figure 5 shows a marked release effect. The stimulus was a series of

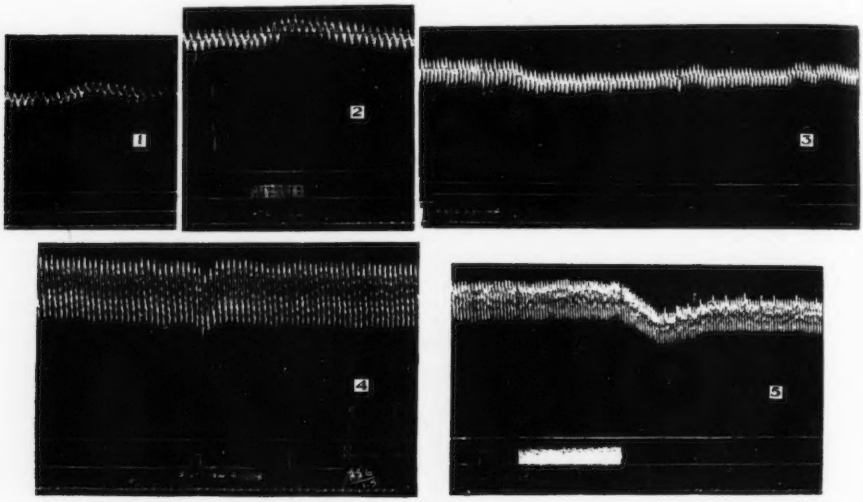

Fig. 1. Blood pressure response to acoustic stimulation. A rise in blood pressure of 7 mm. of mercury followed a stimulus at a frequency of 1024 each second, lasting 2 seconds.

Fig. 2. A rise in blood pressure of 6 mm. of mercury during a series of repeated sounds at a frequency of 360 each second.

Fig. 3. A fall in blood pressure of 6.5 mm. of mercury during a prolonged acoustic stimulation with 8000 vibrations each second lasting five minutes.

Fig. 4. A fall in blood pressure of 5 mm. of mercury during an acoustic stimulation with 1000 vibrations each second, lasting six seconds.

Fig. 5. A fall in blood pressure of 9 mm. of mercury following the cessation of a series of short repeated sounds of 256 vibrations each second lasting one minute and twenty-five seconds.

short sounds at 256 vibrations each second. The same frequency, when previously employed as a stimulus of single, prolonged sounds, failed to elicit any response. At the cessation of the series of repeated sounds, however, a fall in pressure of 9 mm. of mercury occurred, followed by a gradual return to normal.

Although the animals were deeply anesthetized, it is not possible to estimate the exact part played by the cerebral arcs in these reactions. For

this reason, it will be necessary to compare these results with the results of similar experiments in normal and decerebrate animals.

SUMMARY

A brief report is made of the effect of acoustic stimulation on the blood pressure of urethanized dogs. Although very decided changes occur, conclusions are not drawn as to the nature of these responses. A number of characteristic tracings are shown.

A FURTHER STUDY OF THE NERVOUS CONTROL OF THE PYLORIC SPHINCTER

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PART I. THE ACTION OF EPINEPHRIN ON THE MAMMALIAN PYLORIC SPHINCTER IN SITU. The action of epinephrin on the pyloric sphincter of mammals has, apparently, never been made the subject of a systematic investigation. Such observations as have been reported were incidental to a more general study of the sympathetic innervation of the sphincter or of the physiologic effects of epinephrin. There is a lack, not only of adequate observations, but also of agreement in the reported results. Such inferences as may be drawn from known facts regarding the function of the thoraco-lumbar sympathetic fibers supplying the sphincter throw little light on the problem because all of the authors who have studied the subject in recent years (Thomas and Wheelon, 1922; Gruber, 1922; Carlson and Litt, 1924) agree that the splanchnic nerves supply both motor and inhibitory fibers to the sphincter. Carlson and Litt brought out the interesting fact that the sphincter responds to splanchnic stimulation with contraction when in a state of low tonus and with relaxation when the tonus is high. In a preliminary report of the present study the author (Thomas, 1926) described a similar relationship between the tonus of the pyloric sphincter and the reaction to epinephrin.

The older observations bearing on the sympathetic innervation and the action of epinephrin on the gastro-intestinal muscle in general have been adequately reviewed in the first three reports mentioned above. The communications mentioned below have to do with the action of epinephrin on the pyloric sphincter in situ. Literature dealing with the excised sphincter will be discussed in part II of this communication. Elliott (1905) observed contraction of the intact pyloric sphincter of the rabbit when epinephrin was administered. Thomas and Wheelon (1922) in the course of an investigation of the innervation of the pyloric sphincter of the dog tried the effect of epinephrin in a few experiments and observed only "pronounced inhibition." Carlson and Litt (1924) in reporting a study

[1] The apparatus described in this part of the paper was designed and some of the experiments on dogs were performed in the Laboratory of Physiology of St. Louis University School of Medicine.

of the reflex control of the pylorus remarked that "It will be remembered that epinephrin induces contraction of the pylorus." They stated further that they had confirmed this result in the dog, but did not describe their experiments with epinephrin in detail.

METHODS. The apparatus used in a majority of these experiments to record the tonus and contractions of the pyloric sphincter in situ contains some original features and possesses certain advantages over the devices previously employed for this purpose. Essentially, it provides a means of measuring and recording the pressure necessary to maintain a constant

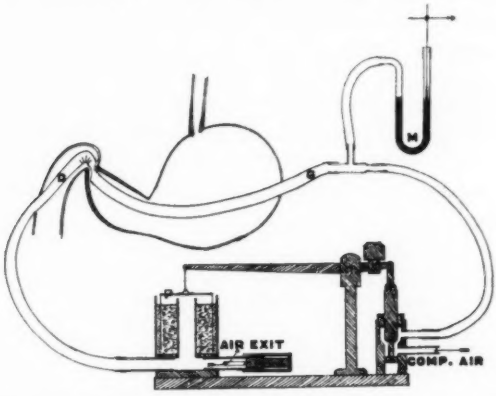

Fig. 1. Diagram of the mechanism used to record changes in tonus and contractions of the pyloric sphincter. The position of the parts is that taken when the apparatus is idle. When in use air under constant high pressure enters the tube labeled "compressed air" in the direction of the arrow, passes through the tube *G* to the pyloric canal and after overcoming the resistance at this point due to the tonus of the sphincter, enters the tube *D* and finally escapes through the slit labeled "air exit." The lateral pressure in the outflow tube raises the piston, *P*, and partially closes the valve, *V*, which regulates the variable pressure in the tube *G* so as to maintain a constant low pressure in the system beyond the pyloric canal. For further description see the text.

flow of air through a flexible tube placed in the pyloric canal. The mechanical principles employed and the arrangement of the apparatus when in use will be evident from a study of the accompanying diagram (fig. 1). The essential parts of the apparatus are: 1, a continuous series of tubes, *G* and *D*, passing through the stomach, pyloric canal and first part of the duodenum, through which a current of air is made to flow. The tubing includes a flexible section which lies in the pyloric canal and is easily compressed by the contraction of the sphincter muscle; 2, a valve, *V*, through which the compressed air passes before entering the gastric end, *G*, of the above system of tubing, the opening and closing of which is con-

trolled by the air pressure in the duodenal or outflow section of the tubing, *D*. The arrangement is such that an increase in the pressure of the escaping air diminishes the size of the inlet valve opening and vice versa; 3, a manometer, *M*, measuring the lateral pressure in the gastric portion of the tubing, i.e., the air pressure on the gastric side of the sphincter.

The air valve is operated by a lever attached to a piston, *P*, the movements of which are controlled by the lateral pressure in the tubes comprising the outflow system, transmitted to the piston through a side opening in a part of the outflow tube built into the base supporting the oil bath in which the piston operates. The air that passes through this opening is confined beneath the piston while the escaping air passes on to an outlet, the area of which is kept constant during an experiment, although an adjustment is provided. Thus the volume of air that passes the pylorus is automatically kept constant and the pressure in the tubes on the gastric side always exceeds by a fixed amount the resistance at the pyloric sphincter and rises and falls with changes in this resistance, consequently a record of this pressure furnishes a quantitative measure of the changes in pyloric resistance. This apparatus will be referred to as the pressure tonometer.

The tubing for the flexible section to be used in the pyloric canal was made of collodion having the following composition:

U. S. P. Collodion	100 parts
Ethyl acetate	15 parts
Castor oil	8 parts

The use of ethyl acetate, which imparts toughness to the membranes, was suggested by the formula published by Looney (1922).

The distal section of the rubber tubing was molded to the curvature of the duodenum by cutting away part of the rubber on one side and, while the desired curvature was maintained with sutures, reëstablishing the continuity of the wall of the tubing with collodion. This precaution made it possible to reduce the length of the flexible section in the pyloric canal to one or two centimeters without danger of its lumen being altered by kinking. The arrangement of tubes in the stomach, pyloric canal and duodenum is a modification of that used by Carlson and his collaborators and was adopted after a study of their work (Carlson, Boyd, and Pearcy, 1922; Carlson and Litt, 1924).

For recording the pressure a mercury manometer, chloroform manometer, or water manometers used singly or connected in series, were used. The chloroform and water manometers were used for smaller animals and for those in which the tonus of the sphincter was low. When a mercury manometer was not used the actual recording was done with a piston recorder connected to the manometer and the records calibrated by direct comparison with a mercury manometer.

The balloon method described by Wheelon and Thomas (1921) was used in some of the experiments. In others the arms of a Cushney myocardiograph were attached to the outer wall of the pylorus over the sphincter and its movements recorded, either in the usual way by means of threads passing over pulleys to a muscle lever, or by a piston recorder through air transmission from a receiving tambour so arranged that approximation of the arms of the myocardiograph compressed the air within it.

Experimental procedure. Nineteen dogs, six cats and eight rabbits were used in this series of experiments. The operations were performed and the experimentation begun under ether anesthesia. When the experiments were prolonged the administration of ether was discontinued after a time and intravenous injections of morphin substituted. Morphin causes marked and sustained contraction of the pylorus and was therefore not used as part of the initial anesthesia. The animals were not starved and reached the operating table for the most part in various stages of gastric digestion, a few with empty stomachs.

Deep surgical anesthesia was maintained during the operation in order that the pylorus might be sufficiently relaxed to permit passage of the tubes without injury. When the pressure tonometer was used the operation consisted of opening the abdomen, making a small opening in the stomach near the cardia and one in the duodenum about ten centimeters from the pylorus, placing the tubes through these openings, and closing the incisions. The openings in the stomach and duodenum were closed with purse-string sutures around the tubes and the abdominal incision was closed with large hemostats. The gastric and duodenal tubes were brought out through slits in the lateral abdominal walls placed so as to avoid kinking the tubes or exerting traction on the viscera.

The technique of placing balloons has been described by Wheelon and Thomas (1921).

In the experiments in which the abdomen was closed with clamps it appeared to be possible that some of the variations in the records were due to changes in intra-abdominal pressure. To control this factor, a number of experiments were performed with the abdominal clamps off and the viscera covered with moist cotton. No results are reported which were not obtainable under these circumstances.

All drugs were given intravenously. The epinephrin preparation used in most of the experiments was the familiar 1–1000 solution of "adrenalin chloride" which contains chloretone. However, each new result was duplicated with solutions freshly prepared from the dry powder containing no preservative.

As soon as the operations were completed the deep surgical anesthesia was discontinued and the animals given just enough ether to keep them quiet. Under these circumstances gastric motility generally begins to

return in the course of one-half to one hour. However, experimentation was begun at once in order to obtain results from the relaxed as well as the active stomach.

EXPERIMENTAL RESULTS. A. *Experiments on dogs.* The experiments of Thomas and Wheelon (1922) by the balloon method were repeated. The results confirmed their observation that epinephrin causes primary relaxation of the sphincter when this method of registration is used. The

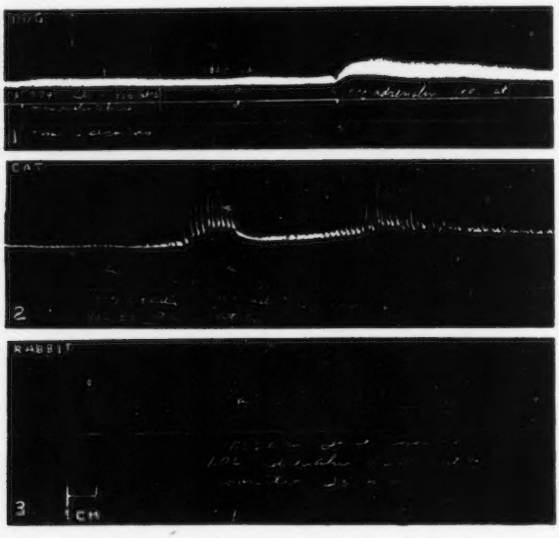

Fig. 2. The action of epinephrin on the relaxed pyloric sphincter. 1. From an experiment on a 15 kilo dog. Record with the pressure tonometer and mercury manometer. The small oscillations are due to the respiratory movements. 2. From an experiment on a 3.3 kilo cat. Record with the pressure tonometer and piston recorder in series with water manometers. Three millimeter amplitude equal 1 mm. Hg. 3. From an experiment on a 1.65 kilo rabbit. Record with pressure tonometer and piston recorder in series with chloroform manometer. Magnification 3.7 x mm. Hg.

results obtained with the myocardiograph corresponded in every way to those described below in connection with the pressure tonometer and need not be discussed separately.

The experiments with the pressure tonometer brought out the fact that the character of the response of the pyloric sphincter to epinephrin is materially modified by experimental conditions. Two factors in particular, namely, the degree of contraction or tonus of the muscle and the size of the dose proved to be of primary importance. These conditions have

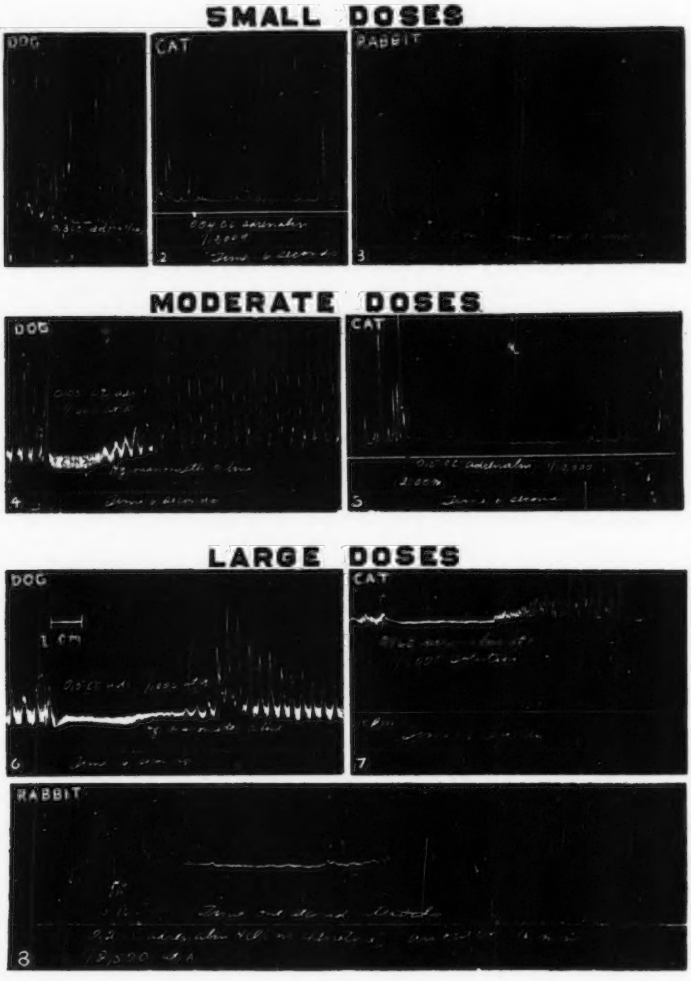

Fig. 3. The action of epinephrin on the active pyloric sphincter. No. 1 is from an experiment on a 13.2 kilo dog, nos. 2 and 5 from a 3.1 kilo cat, no. 3 from a 1.65 kilo rabbit, nos. 4 and 6 from an 11.8 kilo dog, no. 7 from a 2.4 kilo cat, and no. 8 from a 1.4 kilo rabbit. All the records were made with the pressure tonometer except no. 7 which was made with a myocardiograph attached to the pylorus. A mercury manometer was used for all the dog and cat records except no. 7. The rabbit records were written with a piston recorder in series with a chloroform manometer and have an amplitude 3.7 times a corresponding mercury manometer record.

previously been shown to influence the behavior of the sphincter under other circumstances (Carlson and Litt, 1924, and Gruber, 1922).

Immediately after the operation, while the sphincter was relaxed and rhythmic contractions were absent, small doses of epinephrin were without effect whereas large doses caused a moderate increase in tonus lasting for several minutes (fig. 2, no. 1). Under these conditions the best responses were obtained with doses between 0.1 and 1.0 cc. of the 1–1000 solution (0.01 to 0.1 mgm. per kilo). If the tonus of the sphincter was raised and rhythmic contractions started, by the administration of stimulating drugs (morphin or pilocarpin) or by stimulation of the vagus nerve, or if it was allowed to recover spontaneously after the operation the predominant characteristic of the response changed from contraction to inhibition. The response under these circumstances occurred in several phases, the number and relative magnitude of which were influenced by the size of the dose.

When the sphincter was active small doses (0.001 to 0.005 mgm. per kilo) were followed by a slight decrease in tonus and cessation or decrease in amplitude of rhythmic contractions, the duration and magnitude of the response being roughly proportional to the size of the dose. The effect lasted from a few seconds to one or two minutes, after which the muscle returned to normal (fig. 3, no. 1).

Moderate doses (0.005 to 0.01 mgm. per kilo) caused a somewhat greater and more prolonged primary inhibitory effect on tonus and contractions which was generally followed after two or three minutes by a secondary effect consisting of a moderate increase in tonus above the normal and a pronounced increase in the strength of the rhythmic contractions (fig. 3, no. 4).

Large doses (above 0.01 mgm. per kilo) were followed by a response similar to the above except that it was complicated by a tendency toward a primary increase in tonus which coincided in point of time with the early part of the inhibitory response, limiting its extent and delaying the relaxation (fig. 3, no. 6). This primary motor phase came more and more into prominence as the dose was increased and in rare instances it completely overshadowed the inhibitory effect on tonus. The rhythmic contractions, however, were always completely inhibited during the early stages of the response to epinephrin in moderate or large doses. Generally, even with large doses the inhibitory effect outlasted the motor effect and the tonus usually fell to below the previous normal before the rhythmic contractions reappeared. The primary reaction was followed, as in the case of moderate doses, by a secondary increase in tonus and an augmentation of the rhythmic contractions lasting for five or ten minutes.

B. *Experiments on cats.* The response of the cat's pyloric sphincter to epinephrin was, in general, like that of the dog except that they were less pronounced and were less frequently characterized by a primary motor

phase. Primary contraction was rarely, though occasionally, seen when the sphincter was not active but never when the tonus was high and rhythmic contractions were present. See figure 3, nos. 2, 5, and 7. The secondary motor effects occurred with the same regularity as in the dog. The cat's sphincter offered greater resistance to the passage of the tubes and appeared to maintain a greater tonus, even when not actively contracting, than that of the dog, and this fact may account for the scarcity of primary motor responses.

The motor effect pictured in figure 2, no. 2, coincides in point of time with the secondary contractions that were constantly present following large doses of epinephrin. There was practically no primary effect, which was the usual result in the cat with low tonus of the sphincter. This curve is reproduced partly because of the interesting effect of the second dose given during the motor phase of the response to the first dose. The contraction of the sphincter following the first dose was doubtless directly or indirectly due to epinephrin, nevertheless, it served as do contractions from all other causes so far studied, to bring out the primary inhibitory effect of the next dose of epinephrin. McSwiney and Brown (1927) reported a similar reversal of the epinephrin effect by epinephrin contraction in excised muscle from the fundus of the rabbit's stomach.

C. *Experiments on rabbits.* The responses in the rabbit were subject to the same influences and manifested the same phases as in the dog, but the motor phases of the reaction were so much more pronounced that the characteristic response should be described as motor rather than inhibitory.

The relaxed, quiescent muscle either failed to respond to epinephrin or responded with contraction, generally much more pronounced than in the dog (fig. 2, no. 3). When the muscle was active, very minute doses, (0.003 mgm. per kilo or less) sometimes caused pure relaxation with decrease or cessation of rhythmic contractions, and generally without evidence of secondary motor effects (fig. 3, no. 3). Larger doses were regularly followed by both primary and secondary motor effects which increased in prominence with increasing dosage (fig. 3, no. 8). Following very large doses (0.03 mgm. per kilo or more) the primary contraction approached or even exceeded the proportions of the spontaneous rhythmic contractions that occur with peristalsis. Following the primary contraction the tonus fell, rhythmic contractions being absent meanwhile, to a level which was sometimes above, sometimes below, and frequently the same as the previous tone level. The secondary effect was similar to that described as occurring in the dog but generally more pronounced.

These results are discussed, along with those described in the second part of this paper, at the end of that section.

PART II. THE ACTION OF CERTAIN DRUGS OF THE PERIPHERAL AUTONOMIC GROUP ON THE EXCISED PYLORIC SPHINCTER. The excised

pyloric sphincter of mammals has not been studied extensively and the few experiments that have been done have met with little success so far as may be determined from the published reports. Smith (1918) studied the action of drugs on the excised pyloric sphincter of various animals and reported that the sphincter, when excised, went into a state of sustained contraction and responded poorly to stimulating drugs. In response to epinephrin, the pyloric sphincter of the cat, rabbit, and human subject he observed to contract "slightly in a few instances while in most instances no reaction could be obtained." Apparently he observed some stimulating effects on the sphincter with pilocarpin because he specifically includes the pyloric sphincter with the other gastric muscle in describing the contractions caused by this drug. Atropin antagonized the effects of pilocarpin.

Brown and McSwiney (1926a) observed only inhibition of the pyloric sphincter of the dog and cat in response to epinephrin, and excitation in response to pilocarpin. The drugs, however, affected only the rhythmic contractions and caused no change in the resting length of the muscle. Epinephrin was without effect on the quiescent sphincter, and manifested no stimulating effect either during or after the action of the drug. They also noted the antagonism of atropin for the effects of pilocarpin. In a later communication (Brown and McSwiney, 1926b) they reported similar results on the rabbit.

I have at various times attempted similar experiments, using a modified Magnus technique that has proved successful in the study of excised intestine. These efforts were attended with such little success that the conclusion was reached that results obtained with the excised sphincter by the usual methods have little significance, unless it be to show that the muscle is not adapted to study by the means generally employed for experiments with excised gastro-intestinal muscle.

Experiments with the pyloric sphincter of cold blooded animals have given more definite results. Gruber (1922) reported that the excised sphincter of the frog was contracted by weak solutions of epinephrin (1–100,000,000) and relaxed by stronger solutions (1–100,000).

METHOD. *Principles of the method.* A valuable hint regarding the probable cause of the frequent failure of the excised pyloric sphincter to react to drugs was furnished by the observations of Šiaulis and Sollmann (1927) who found that the circular muscle of the excised intestine failed to function and manifested signs of asphyxia unless special precautions were taken to protect the tissue from asphyxia while it was being prepared and to secure good contact of the muscle with the solution.

Another difficulty that has long been recognized has recently been studied extensively by Sollmann, von Oettingen and Ishikawa (1928). This is the loss of carbon dioxide and consequent change in hydrogen ion

concentration with aeration or oxygenation of bicarbonate buffered solutions such as Locke's solution. This difficulty becomes especially serious when the period of observation is prolonged as it necessarily is with the pyloric sphincter because it recovers its irritability very slowly after excision even under the most favorable conditions.

The procedure that was finally adopted for the study of the excised pyloric sphincter was designed to secure more adequate oxygenation of the immersion bath than has hitherto been considered necessary, to provide for regulation of the carbon dioxide and therefore of the hydrogen ion concentration of the bath without altering the concentration of other constituents of the solution and to prevent deterioration of the tissue after excision while it was being prepared for study.

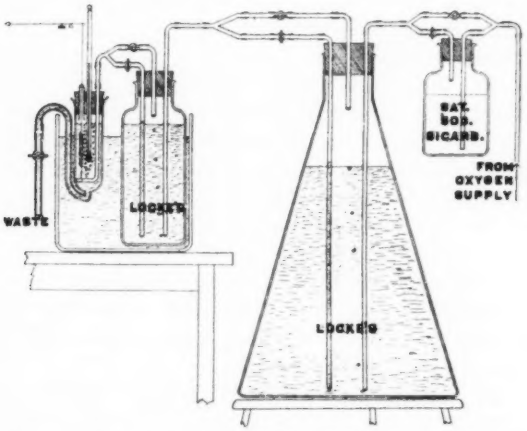

Fig. 4. Diagram of the apparatus used for the study of the excised pyloric sphincter. The temperature of the water bath was regulated by means of an electric thermoregulator and heater not shown in the diagram. For explanation see text.

Apparatus. The arrangement of the apparatus is indicated in the diagram (fig. 4). Three vessels were provided containing Locke's solution, two of which were kept in a water bath maintained at a temperature of about 38 degrees Centigrade by means of an electric thermoregulator and heater. One of these contained the bath for the muscle and the other a supply of warm Locke's solution for refilling the bath when necessary. The third was outside the water bath and contained a large volume of Locke's solution at room temperature. The three vessels were connected to one another by tubes arranged so that oxygen could be bubbled through the three solutions in succession, beginning with the cold solution, or by turning stopcocks, solution could be forced by the pressure of the gas from

the large container outside the water bath into the vessel containing the reserve supply of warm solution, or separately, from the latter into the vessel for the muscle bath. The muscle bath was emptied when necessary through a branch tube leading from the bottom of the vessel and connected to a siphon.

In order to limit the loss of oxygen from the muscle bath by diffusion from the surface the vessel containing it was provided with a stopper through which a single hole remained open for the transmission of the thread that connected the muscle to the muscle lever. Even this opening was partly closed by inserting in it a short piece of glass tubing drawn out at the upper end to a diameter only a little larger than the thread. The continuous outflow of oxygen through the narrow space around the thread probably effectively prevented dilution of the atmosphere of oxygen above the bath with air so that the oxygen tension of the bath remained approximately at one atmosphere.

The object of the arrangement by which the oxygen was passed first through a large volume of solution was to decrease the rate of change of the hydrogen ion concentration of the muscle bath by allowing the oxygen to pick up all the carbon dioxide that it would take from a Locke's solution before it was bubbled through the bath. Even with this arrangement the solutions became noticeably alkaline after prolonged oxygenation and to restore the lost carbon dioxide the oxygen was bubbled for a part of the time through a saturated solution of sodium bicarbonate before passing it through the Locke's solution. As long as the oxygen was being bubbled through the bicarbonate solution the normally alkaline drift in the pH of the oxygenated solutions was replaced by an acid drift. The drift in either direction was slow and had to be reversed only about once every half-hour. The pH of the Locke's solution was observed from time to time colorimetrically in samples taken from the reservoir bottle in the water bath and was permitted to vary only between 7.6 and 7.8.

Procedure. In preparation for an experiment on the excised sphincter a liter or more of Locke's solution was cooled to between zero and four degrees Centigrade and thoroughly oxygenated, and then divided into a larger and a smaller portion which were kept in vessels packed in ice and salt. The animal was then killed and as quickly as possible the abdomen was opened and a portion of the stomach and duodenum including the pyloric sphincter rapidly excised and dropped at once without cleaning into the larger portion of the cold Locke's solution. Rabbits were killed by a sharp blow on the occiput and dogs and cats were quickly overcome by the rapid administration of chloroform and killed by section of the medulla.

After the excised tissue had cooled the lumen was opened by a longitudinal incision and the specimen thoroughly washed in the cold solution and

then transferred to the other vessel of cold Locke's. The sphincter muscle was dissected out with the tissue immersed so far as possible in the cold solution. The mucosa of the pyloric canal was first clipped away with scissors. In the preparations from the dog and cat, the slightly contracted sphincter muscle could then be easily distinguished standing out like a partially buried whipcord at the junction of the gastric and duodenal muscle. It was dissected out and freed so far as possible from all adherent muscle and connective tissue.

In the dog and cat the satisfactory isolation of the sphincter muscle presents no difficulties, but in the rabbit the situation is quite different. After clipping the mucosa it is easy to see where the stomach ends and the duodenum begins but no cord-like sphincter is evident. Instead, the gastric muscle thickens gradually to the end and it is wholly a matter of judgment how much one cuts away and calls the sphincter. In these preparations the duodenal muscle was completely removed and then a band of muscle no wider than its thickness was cut from the free pyloric end of the stomach. Probably most of the sphincter muscle was included in this band and certainly not much more.

When the dissection was finished threads were attached to the cut ends of the open ring of sphincter muscle with which it was immediately attached to the apparatus and placed in the previously oxygenated and warmed bath.

The irritability of the muscle gradually increased for four or five hours after it was placed in the bath. The preparations failed completely to respond to drugs at first and generally only feeble reactions could be obtained during the first hour.

Drugs were dropped into the bath from a Record syringe calibrated in hundredths of a cubic centimeter, through a hypodermic needle that was pushed through the stopper and left in place. The drugs used were "adrenalin chloride, 1–1000" and "adrenalin" in powder form (Parke-Davis) pilocarpin hydrochloride, and atropin sulphate (Merck). All were prepared in 1–1000 solution in Locke's (except the stock 1–1000 adrenalin which is in saline) from which appropriate dilutions were made with Locke's solution for convenient measurement of the various amounts to be used. The volume of the bath was 80, 90 or 100 cc. (always a known amount) and the concentrations attained in the bath were calculated from the volume of solution and the amount of drug added, without correcting for the volume of liquid added with the drug, which was never more than 1 cc. Records were made with a muscle lever and kymograph in the usual way. The lever was weighed so that 2 grams added to the weight of the string and immersed muscle just balanced it.

Due attention was paid to the possibility that the preservative (chloretone) in commercial adrenalin solutions or slight changes in pH due to

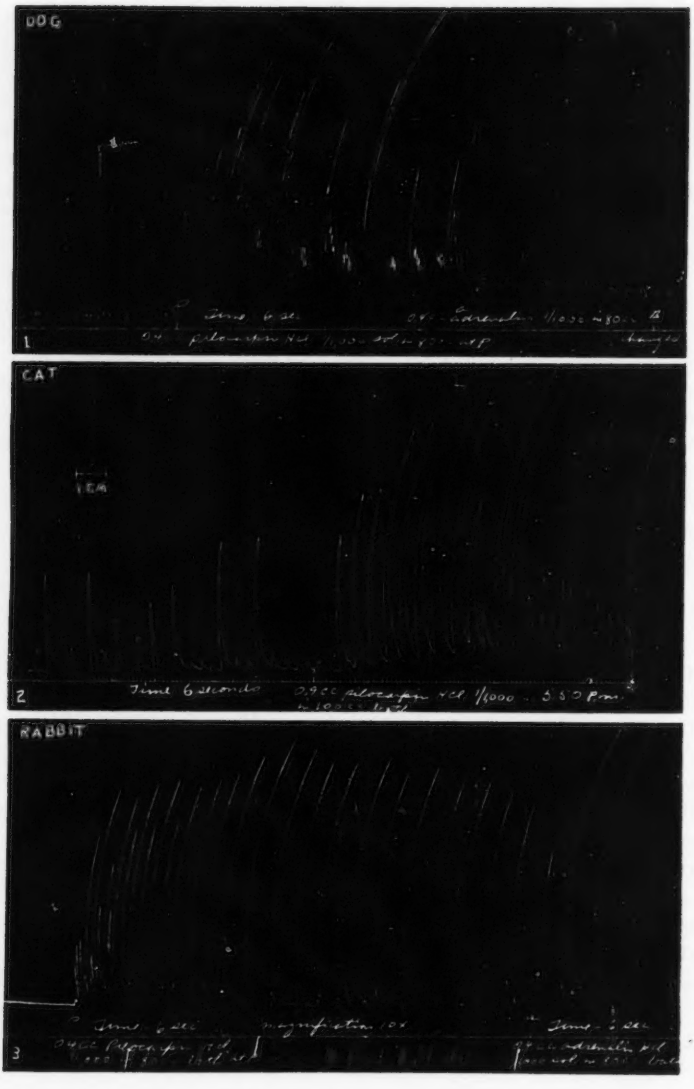

Fig. 5. The action of pilocarpin on the excised pyloric sphincter of the dog (1), cat (2), and rabbit (3).

the drug solutions might influence the results. Each type of response to epinephrin was duplicated with solutions of the pure substance made neutral or alkaline just before they were added to the bath. No constant difference was seen between the effects of slightly acid or slightly alkaline drug solutions or of solutions with or without chloretone.

RESULTS. This report comprises the results of experiments on seventeen excised sphincters, ten of which were from rabbits, five from cats and two from dogs. Particular attention was given to the effects of epinephrin and pilocarpin but some observations were also made with atropin and barium chloride.

The effects of pilocarpin. In all irritable preparations, regardless of their source, pilocarpin caused a moderate increase of tonus and greatly augmented the rhythmic contractions if they were present or initiated them if the muscle was inactive (fig. 5). Before the preparations had completely recovered their irritability and in some that never became very responsive to drugs it initiated rhythmic contractions without causing any change in the resting length (tonus) of the muscle. The amount of drug employed was such as would make of the bath a 1–200,000 or 1–400,000 solution of pilocarpin hydrochloride.

The effects of epinephrin on the dog's sphincter. The following description is based on the reactions of the more irritable of the dog sphincters. The other preparation reacted to the higher concentrations in a qualitatively similar manner but it did not react to the more dilute solutions. The more reactive preparation responded to epinephrin in very dilute solutions (1–80,000,000) with a slight contraction not followed by relaxation beyond the previous normal (fig. 6, no. 1). Higher concentrations (1–8,000,000) caused a more marked contraction followed by an approximately equal relaxation beyond the normal resting length of the muscle. With still higher concentrations the primary contractions occurred as usual while the relaxations became more and more pronounced and prolonged, assuming very definitely the predominant rôle in the reaction. Increasing the tonus of the muscle by application of pilocarpin did not alter the response to epinephrin except to further accentuate the relaxation (fig. 6, no. 2). However, when the muscle was strongly contracted by means of the application of barium chloride, the primary contraction no longer appeared following the application of epinephrin, the reaction consisting merely of pronounced relaxation. Rhythmic contractions, if present, were always inhibited by epinephrin except following barium chloride, when they frequently persisted even at the much lower tone level established by the epinephrin.

The effects of epinephrin on the cat's sphincter. The characteristic reaction of the cat's sphincter was a slight but prolonged relaxation (fig. 6, no. 3). Some of the preparations maintained a fixed resting length in spite

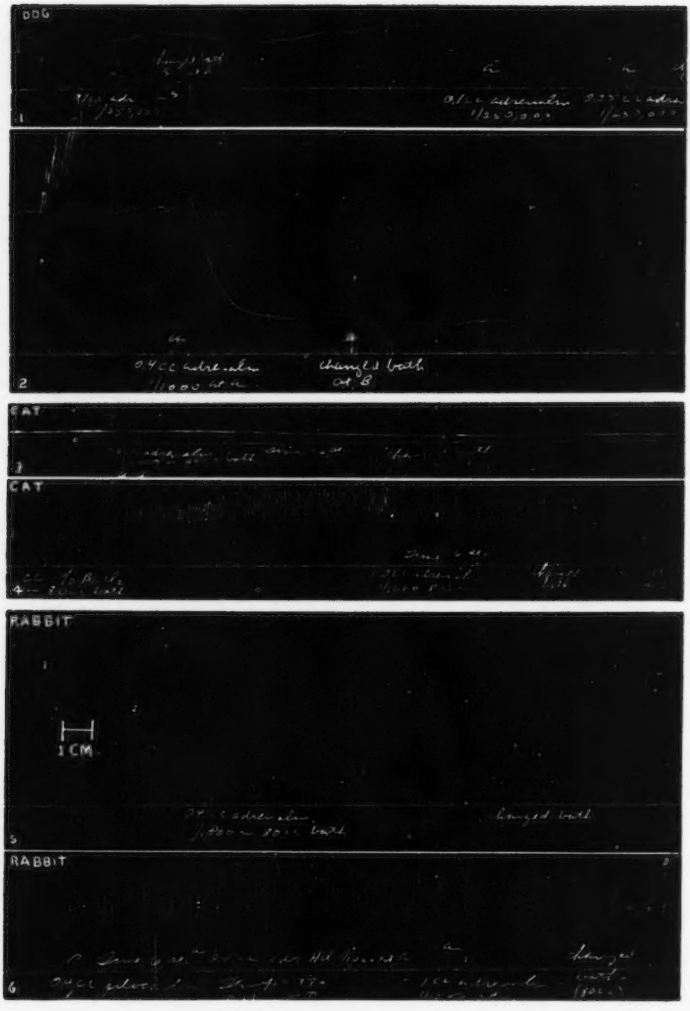

Fig. 6. The action of epinephrin on the excised pyloric sphincter.

1. The effect on the dog's sphincter of minute doses. (The contraction was followed by relaxation when larger doses were used.)

2. The effect on the dog's sphincter following pilocarpin. The contraction due to pilocarpin accentuates the epinephrin relaxation.

3. Typical effect observed on the cat's sphincter when not previously stimulated by drugs.

4. Relaxation produced in the cat's sphincter during barium contraction.

5. Typical effect on the unpoisoned rabbit sphincter.

6. Typical effect on the rabbit's sphincter following pilocarpin. The first contraction is due to pilocarpin.

ot the application of drugs and those that did manifest changes in tonus responded very slowly. When contracted by means of barium chloride they relaxed somewhat more readily in response to epinephrin (fig. 6, no. 4) but even then the reaction was slightly compared to that seen in the dog's sphincter.

The effects of epinephrin on the rabbit's sphincter. With one exception, the preparations from the rabbit responded to epinephrin with contraction, regardless of the concentration of the drug or the previous state of the muscle (fig. 6, nos. 5 and 6). Usually the extent of the contraction increased with the concentration of epinephrin up to about 1–200,000. The contractions were of considerable extent, frequently representing several millimeters actual change in the length of the muscle. The rhythmic contractions, when present, were frequently augmented by the epinephrin but slowed somewhat in rate, about as frequently they were inhibited.

In the single exceptional instance mentioned above, the muscle responded like the others when relaxed and quiescent, except that the contractions in response to epinephrin were less pronounced. However, after pilocarpin had been administered and the muscle contracted somewhat and rhythmic movements begun the responses to epinephrin resembled those of the cat's sphincter, i.e., there was inhibition of the rhythmic contractions, sometimes with and sometimes without loss of tonus.

The effects of atropin. The effects of atropin were tried in one experiment on the dog's sphincter and in several of those from the rabbit. In all cases it caused cessation of rhythmic contractions and a gradual loss of tonus. The concentrations employed were, 1–800,000 and 1–400,000 on the rabbit's sphincter and 1–200,000 on the dog's sphincter. The effects of pilocarpin and epinephrin following atropin were studied on the rabbit's sphincter. Atropin had its usual effect of completely abolishing the response to pilocarpin. In these experiments a 1–800,000 solution was sufficient for this purpose. However, the epinephrin contraction appeared as usual in the presence of atropin in 1–400,000 solution.

The effects of barium chloride. Barium chloride (1–10,000 or more) caused an immense shortening of the muscle in all cases and initiated powerful rhythmic contractions. These effects, in contrast to the similar effects of pilocarpin, passed off very soon after the barium was removed by washing the muscle with fresh solution.

DISCUSSION. The reactions of the pyloric sphincter in situ to epinephrin may be said to follow the same general plan in all three species, namely, contraction when the sphincter is relaxed and relaxation when it is contracted. However, such a generalization perhaps involves giving more consideration than is warranted to the contractions of the cat's sphincter and the relaxations of the rabbit's sphincter which were produced

but rarely and under extreme conditions. Certainly the much more constant and marked differences that appeared in the reactions in the different species cannot be wholly disregarded and indicate at least quantitative differences in the relation between motor and inhibitory components in the mechanism that reacts to epinephrin.

The apparent discrepancy between the results obtained in the dog by Carlson and Litt (1924) and those reported by Thomas and Wheelon (1922) disappears in the light of these experiments. The constant occurrence of inhibitory responses to epinephrin in the experiments of the latter authors was evidently due to their practice of waiting for gastric motility and tonus to recover after the operation before beginning observations and the inhibition was probably accentuated by a stimulating effect of their type of balloon on the pyloric tonus mechanism. Carlson and Litt, on the other hand, were probably observing a relaxed sphincter when they tried the effect of epinephrin.

Contraction of the pyloric sphincter of the rabbit in response to epinephrin as described by Elliott (1905) is verified by these experiments. Although relaxation of the rabbit's sphincter is obtainable with this drug under special conditions, contraction is beyond doubt the characteristic response. It seems a little unfortunate that Elliott should have chosen the rabbit for his experiments since it appears that his observation has determined the prevalent conception of the reaction, and the rabbit is the only one of the three common laboratory animals in which contraction of the sphincter in response to epinephrin occurs with sufficient regularity under a variety of conditions to warrant describing the motor effect as characteristic.

The reactions to epinephrin described by Smith (1918) are confirmed only for the rabbit's sphincter. He observed either contraction or no effect in the excised cat's sphincter whereas in these experiments the effect was characteristically inhibitory in irritable preparations. His results with pilocarpin and atropin, insofar as they are described in his report, are confirmed. Insofar as Brown and McSwiney (1926a) were able to elicit the effects of drugs on the excised sphincter of the cat and dog their results are confirmed in every detail, but with the addition of characteristic effects on tonus which they failed to observe. The results with epinephrin on the excised rabbit sphincter differed from theirs (1926b) in that occasional stimulating effects on the rhythmic contractions made their appearance and instead of no effect on tonus a definite motor effect was demonstrated.

The observation by Gruber (1922) on the frog's pyloric sphincter of contraction in response to small amounts of epinephrin and relaxation when larger amounts were used corresponds to the results obtained with a single preparation from the dog. The other experiments cannot properly be

regarded as in conflict with his results inasmuch as the concentrations with which he obtained contraction were without effect on most of the preparations and therefore the experiments on the excised sphincter must be regarded as inconclusive with regard to the effects of very dilute solutions.

Except for minor quantitative differences the reactions of the pyloric sphincter in situ correspond to the reactions that have been described as accompanying stimulation of the thoraco-lumbar sympathetic nerves. The pyloric sphincter may therefore be included with those organs that react in the same way to epinephrin as to sympathetic stimulation. Consequently the experiments with epinephrin on the excised sphincters may properly be used to confirm and extend our knowledge of the sympathetic innervation. They have the advantage over experiments on the sphincter in situ of eliminating the indirect effects of reactions in adjacent organs, changes in the blood supply, the effects of anesthesia, and other responses of the organism as a whole to the operative and other experimental procedures. Furthermore the reactions of the excised sphincters were more constant than those of the sphincter in situ and were relatively independent of changes in the tonus of the muscle. Furthermore, they correspond in detail with the primary responses to moderate and large doses of epinephrin of the sphincters of the same species in situ when in a state of moderate tonus, that is, the reactions that were most readily obtained under average conditions. The reaction obtained consistently in the excised preparations and under ordinary conditions in the sphincters in situ are: contraction in the rabbit, relaxation in the cat and slight contraction followed by more pronounced relaxation in the dog.

From these results the conclusion appears to be justified that the sympathetic innervation governing the tonus of the pyloric sphincter is predominantly motor in the rabbit and, though less definitely, inhibitory in the dog and cat. The existence of motor components in the sympathetic innervation to this region in the dog and cat has been repeatedly demonstrated and their tendency to largely counterbalance the inhibitory components may account for the difficulty of obtaining marked reactions to epinephrin in these two animals, particularly in the cat.

Inhibition of the rhythmic contractions by epinephrin was much more marked than inhibition of tonus. It occurred without exception as a primary effect in all of the experiments on the sphincter of the dog and cat whether excised or in situ, and, except in some of the excised material, in the rabbit. This suggests that the sympathetic innervation governing the rhythmic contractions differs from that governing tonus and is predominantly inhibitory in all three animals but comprises demonstrable excitatory components in the rabbit.

The tendency of recent work, particularly that of Carlson, Boyd, and

Pearcy (1922) and Carlson and Litt (1924) has been to indicate that the conception of the extrinsic nerves as consisting of some fibers that are constantly motor in function and others constantly inhibitory is not adequate to explain the varied reactions that may be obtained when these nerves are stimulated. Carlson (with Boyd and Pearcy, 1922) suggests that they may more properly be regarded as analogous with association fibers between reflex centers. While admitting the value of this conception as applied to fibers that terminate in relation to the intrinsic plexus I find it difficult to apply to fibers that terminate directly on muscle, as is believed to be the case with postganglionic sympathetics, and more especially to the terminal mechanism on which epinephrin acts. Probably Carlson had no thought of applying it to these structures. At any rate the customary conception of the terminal mechanism as definitely motor or inhibitory has not yet been proven inadequate and I do not regard the above discussion of the distribution and relative effectiveness of the components of such mechanism as out of harmony with the conception of the innervation proposed by Carlson when that conception is limited, as it must be, by structural considerations.

In the above discussion the secondary motor effect which was characteristic of the reaction of the sphincter in situ to epinephrin in all three species has been disregarded since nothing resembling it appeared in any of the excised preparations. Probably its appearance in the reactions of the sphincters in situ was due to an indirect effect of changes in other organs rather than to a stimulating action of epinephrin on the pyloric motor mechanism. This conclusion is based partly on its consistent absence from the reactions of the excised sphincters and partly on the fact that no similar effect (increase in both tonus and rhythmic contractions) ever appeared as a primary reaction in the sphincters in situ. Frequent unsuccessful efforts were made to produce such effects by the administration of very small doses, by the continuous infusion of dilute solutions or by the administration of a second dose of epinephrin at a time when the secondary effect of a previous dose was due to appear. In the last instance the second dose invariably had the characteristic primary effect of such dose instead of augmenting the secondary effect of the previous dose. One possible cause of the secondary motor effect is depression, following overstimulation by the epinephrin, of inhibitors of gastric peristalsis.

Reversal of the effect of epinephrin on the sphincter with changes in tonus which characterized the reactions of the sphincter in situ was generally not evident in the experiments on the excised sphincter. Such reversal has, however, been demonstrated in excised gastric muscle (Brown and McSwiney, 1926b). The excised preparations used in these experiments differed from those studied by Brown and McSwiney and from the sphincter in situ in that they were free from mucosa and longitudinal

muscle and therefore probably quite free from plexus cells. Although the association of injury to the plexus with the absence of reversal effects may have been coincidental it is at least suggestive of the possibility that changes in the plexus may be primarily responsible for these effects.

SUMMARY AND CONCLUSIONS

1. The action of epinephrin on the pyloric sphincter in situ and of epinephrin, pilocarpin, atropin, and barium chloride on the excised pyloric sphincter has been studied in the dog, cat, and rabbit. Many of the difficulties that have hitherto attended such studies have been obviated by improved methods.

2. The primary action of epinephrin on the pyloric sphincter in situ may be to cause either increase or decrease of tonus in all three species, the former when the muscle is relaxed and the latter when it is contracted. Increase of tonus in the cat and decrease of tonus in the rabbit were produced but rarely. Epinephrin generally inhibited rhythmic contractions.

3. The effects of epinephrin on the excised pyloric sphincters corresponded in detail to the results most commonly produced in the sphincters in situ, and were: slight contraction followed by more pronounced relaxation in the dog, relaxation in the cat, and contraction in the rabbit sphincters.

4. A secondary increase of tonus and augmentation of rhythmic contractions followed the primary effect of epinephrin in the sphincters in situ but not in the excised sphincters. Reasons are given for interpreting this as an indirect effect, not due to the action of epinephrin on the pyloric motor mechanism.

5. The results with epinephrin are interpreted as indicative of a slight preponderance of inhibitory over motor components in the thoracolumbar sympathetic innervation governing the tonus of the pyloric sphincter in the dog and cat and marked preponderance of motor over inhibitory components in the rabbit.

6. Previous observations of the excitatory effect of pilocarpin on the pyloric sphincter and the antagonism of atropin for the pilocarpin effects were confirmed on the excised material. Barium chloride caused marked increase of tonus and augmentation of rhythmic contractions.

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STUDIES ON DIABETES INSIPIDUS

III. The Diuretic Substance, Further Observation[1,2]

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The results of experiments reported in the first and second papers of this series (Bourquin, 1927) led to the conclusion that the diuresis of experimental diabetes insipidus is due to the production of a diuretic substance at the site of effective lesion in response to irritation. The presence of such a diuretic substance was demonstrated in the blood and in extracts of the mammillary bodies and immediately adjacent tissue of the brains of dogs with experimental diabetes insipidus but not in the same quantities of the blood or extracts of that region of the brain of normal dogs.

The present investigation was undertaken to determine whether or not the diuretic substance is present in the urine of dogs with experimental diabetes insipidus and in the mammillary bodies of normal brains, and to study its chemical properties further in order to secure it in more nearly pure form for the purpose of investigating its physiological action.

Methods. The diuretic properties of the preparations to be described were tested by intravenous injection into unanesthetized dogs, which had not received food during the preceding twelve hours. The urine was collected by retention catheter for a control period of from one to one and a half hours before the injection and for from four to seven hours after the injection. The results have been expressed arbitrarily in terms of $\frac{\text{cubic centimeters of urine per hour after injection}}{\text{cubic centimeters of urine per hour before injection}}$ since other modes of calculation lead to the same conclusions.

Urine. The presence or absence of a diuretic substance peculiar to the urine of dogs with experimental diabetes insipidus should be demonstrated by comparing the diuresis produced by preparations of urine secreted by diabetic dogs with that produced by preparations of urine secreted by

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[2] Reported before the Fortieth Annual Meeting of the American Physiological Society.

normal, that is by non-diabetic, dogs under the influence of a water diuresis of the same intensity as the diuresis of the diabetic dogs caused by the administration of water by stomach tube, this precaution being necessary to control the possibility that any changes detected in the blood or urine of the dogs with experimental diabetes insipidus are the results of the excessive ingestion of water and of the diuresis per se (Christie and Stewart, 1917, 1922; Kayser and Breton, 1925). That procedure was followed, the experiments being controlled further by depriving the animals of food for a period of twelve hours preceding the collection of the urine, which was prepared for injection by rendering it just acid to litmus paper with sulphuric acid, evaporating it to a thick syrupy mass, and extracting the latter with 37.5 cubic centimeters of 95 per cent ethyl alcohol per hour's output of urine to remove the toxic substances. The residue was redissolved, neutralized with barium hydroxide, filtered, and adjusted to volume, 5 cc. of the final preparation representing the urine secreted over a period of one hour by a dog weighing 18 to 20 kgm. In testing the diuretic properties of the preparations 5 cc. were injected per 1.5 kgm. of dog.

Extracts from the brains of normal animals. On the same theory the presence or absence of a diuretic substance peculiar to the region of the mammillary bodies of normal brains was tested by comparing the diuretic properties of extracts of the mammillary bodies with the diuretic properties of similar preparations made from equivalent amounts of tissue cut from other portions of the brain.

In the first series of experiments the mammillary bodies were cut from the heads of hogs, cattle, and sheep immediately after they had been slaughtered, were ground to a paste in sand moistened with 0.5 per cent sulphuric acid, and extracted with three to four volumes of acetone three times for periods of four, twelve, and six hours respectively. The acetone was evaporated from the residue, which was ground to a powder, extracted with frequent shaking for from three to four hours in 4 to 5 volumes of 0.2 per cent sulphuric acid, boiled, acidified with dilute sulphuric acid until the maximum precipitation of proteins occurred, filtered, neutralized with barium hydroxide, filtered, and evaporated to a volume of 10 cc. for injection, each preparation representing the extract from the mammillary bodies of two to five hundred brains and each control preparation representing the extract from equivalent amounts of material taken at random but chiefly from the parietal lobes of the cerebral hemispheres.

In the second series of experiments the extracts were prepared in the same way as those of the first series but each injection represented the extract from the lateral walls of the third ventricle and the mammillary bodies of the brains of 480 cattle and the tissue for the control injections was cut from the adjacent thalamic region.

These preparations were also injected into dogs weighing 4.5 kgm.

Chemical properties. Active preparations of diabetic urine were precipitated by phosphotungstic acid and prepared for injection by the usual method, three experiments. In addition the effects of ashing, of lipoid solvents, and of treatment with alkaline solution on the diuretic substance were studied by comparing the diuretic properties of the preparations described above and of active preparations of the blood of diabetic dogs with the diuretic properties of similar preparations, which had been treated further by ashing, or by extraction with acetone, 95 per cent ethyl alcohol or ether, or by treatment with alkali, that is, barium hydroxide.

In the latter group of experiments the blood of dogs prepared by precipitating with phosphotungstic acid was divided into equal portions of twenty cubic centimeters, each of which represented three hundred cubic centimeters of whole blood. One portion was kept as a control. To each of the other portions a saturated solution of barium hydroxide was added drop by drop till it was weakly, moderately, or strongly alkaline in reaction as indicated roughly by indicators the solutions being too highly colored to determine the pH with any degree of accuracy. The solutions were promptly filtered, the precipitates washed with a solution of half saturated barium hydroxide, and the combined filtrates and washings neutralized with sulphuric acid and prepared for injection in the usual way. In a few cases the precipitates were redissolved in distilled water acidified with sulphuric acid and were likewise prepared for injection. As a further control inactive preparations were made by saturating the blood precipitated with phosphotungstic acid with barium hydroxide and letting it stand at room temperature for some hours.

The extracts of the mammillary bodies and walls of the third ventricles were prepared as previously described. A saturated solution of barium hydroxide was then added drop by drop till the extract was adjusted to a specified pH, which was determined by the colorimetric method using thymol sulfonephthalein, phenolsulfonephthalein, and phenolphthalein as indicators and the boric acid potassium chloride-sodium hydroxide mixtures of Clark and Lubs (Clark, 1922) as standards. These solutions were promptly filtered, the precipitates washed with a solution of barium hydroxide of the same pH, redissolved, and prepared for injection. The combined filtrate and washings were neutralized with sulphuric acid and made ready for injection.

All of these preparations were injected into dogs weighing approximately four and a half kilos.

RESULTS. *Urine.* The diuresis produced by preparations of urine secreted by dogs with experimental diabetes insipidus, 14 experiments, was on the average more intense and more prolonged than the diuresis produced by preparations secreted by normal dogs, 11 experiments (table 1). The range of variation in the diuretic activity of the preparations of urine

secreted by normal dogs was relatively small, while that of the preparations secreted by diabetic dogs was materially greater some of the injections producing a diuresis well within the limits of the diuresis produced by injections of normal urine and others greatly exceeding those limits (table 1).

These experiments are interpreted as demonstrating a diuretic substance in the urine of dogs with experimental diabetes insipidus, which is not present in the urine of normal dogs in comparable amounts, and which is apparently labile. It is thought that it was probably destroyed in the course of preparing certain of the urines of diabetic dogs by the pH at

TABLE 1

The diuresis produced by preparations of urine secreted by dogs with experimental diabetes insipidus compared with that produced by similar preparations of urine secreted by normal dogs. The diuresis is expressed in terms of

$$\frac{\text{cc. of urine per hour after injection}}{\text{cc. of urine per hour before injection}}$$

EXPERIMENT	DIURESIS DURING THE FIRST FOUR HOURS FOLLOWING THE INJECTION		DIURESIS DURING THE FIFTH HOUR FOLLOWING THE INJECTION	
	Donner diabetic	Donner normal	Donner diabetic	Donner normal
1	15.104	1.316	13.0	0.904
2	6.428	2.056	3.89	2.89
3	5.501	1.542	3.27	1.03
4	4.749	1.975		1.40
5	3.571	2.479		
6	8.393	1.476	6.8	0.986
7	9.525	2.682	5.2	1.09
8	3.953	4.195	1.23	2.83
9	4.785	2.615	1.5	1.16
10	2.807	2.241	1.25	2.56
11	2.727	5.344	1.01	
12	2.471		0.8	
13	2.111		2.0	
14	10.224		6.41	
Average.....	5.882	2.538	3.78	1.65

which they were concentrated and that this may possibly explain the negative results obtained by the injection of small amounts of urine reported in the preceding paper and the negative results reported by Roussy (1925) following the injection of the urine of diabetic animals.

Extract from the brains of normal animals. The extract from the mammillary bodies of 500 normal animals produced the same degree of diuresis as the extract from the mammillary bodies of 3 to 5 diabetic dogs (Bourquin, 1927b). As in the case of the latter the diuretic substance in the mammillary bodies of normal animals is not distributed uniformly throughout the brain for the urine output was increased by an average of 355.66 per

cent during the first five hours following the injection of the mammillary body extracts, five experiments, and by an average of only 87.2 per cent by the injection of extracts of the same amount of brain tissue selected at random but chiefly from the parietal lobes of the cerebral hemispheres. On the other hand the diuretic substance is not sharply localized in the mammillary bodies for the injection of extracts of tissue from the thalamic

TABLE 2

Protocol illustrating the effects of treating inactive preparations (57 and 34) and active preparations (47 and 33) of blood from dogs with experimental diabetes insipidus with solutions of barium hydroxide. The diuretic action was tested on dog number 6 in each case. The diuresis is expressed in terms of:

$$\frac{\text{cc. urine per hour after injection}}{\text{cc. urine per hour before injection}}$$

EXPERIMENT	CONDITION OF THE DONNER	TREATMENT OF THE PREPARATION BEFORE INJECTION	DIURESIS DURING FOUR HOURS FOLLOWING THE INJECTION	DIURESIS DURING THE FIFTH HOUR FOLLOWING THE INJECTION
4	Normal	None	2.45	1.28
		Ashing	3.006	2.21
57	Diabetes insipidus	None. (Preparation inactive)	1.89	
		$Ba(OH)_2$ solution, medium strength	2.392	2.94
34	Diabetes insipidus	None. (Preparation inactive)	1.742	0.93
		$Ba(OH)_2$ solution, medium strength	1.277	0.90
		$Ba(OH)_2$ solution, weak	1.422	1.45
47	Diabetes insipidus	None. (Preparations active)	4.444	7.63
		$Ba(OH)_2$ solution, strong	6.124	8.6
		$Ba(OH)_2$ solution, weak	2.488	2.44
		$Ba(OH)_2$ solution strong	1.384	1.66
33	Diabetes insipidus	None. (Preparation active)	4.4	5.0
		$Ba(OH)_2$ solution, strong	6.284	7.29
		$Ba(OH)_2$ solution, weak	1.486	1.63
		$Ba(OH)_2$ solution, medium strength	1.971	
		Ashing	1.831	0.798

region, 4 experiments, produced as great a diuresis as the injection of extracts of the combined mammillary bodies and lateral walls of the third ventricle, and the diuresis produced by extracts of the latter, 9 experiments, was proportionately greater than that produced from extracts of the mammillary bodies alone of a like number of animals (table 4).

These results would seem to answer the criticisms made by Trendelenburg (1928) who holds that the diuresis produced by extracts of the mammillary bodies of dogs with experimental diabetes insipidus (Bourquin,

1927b) was obtained because the dogs were anesthetized with sodium barbital. He bases this statement on the fact that he finds that the injection of the extract of 3 mgm. per kilo of the tuber cinereum of normal dogs partially inhibits diuresis in unanesthetized rabbits, that the extract of equivalent amounts of the mammillary bodies of normal dogs produces a questionable slight inhibition of diuresis, and that the extracts of 3 mgm.

TABLE 3

Summary of the results of treating preparations of blood from dogs with experimental diabetes insipidus, which contained the diuretic substance of diabetes insipidus, with solutions of barium hydroxide using as controls preparations of the blood of dogs with experimental diabetes insipidus which did not contain the diuretic substance, and of the blood of normal dogs. The diuresis is expressed in terms of:

$$\frac{\textit{cc. urine per hour after injection}}{\textit{cc. urine per hour before injection}}$$

CONDITION OF THE DONNER	DIURETIC SUBSTANCE IN THE ORIGINAL PREPARATION	TREATMENT OF THE PREPARATION	NUMBER OF EXPERIMENTS	DIURESIS DURING THE FIRST FOUR HOURS FOLLOWING THE INJECTION	DIURESIS DURING THE FIFTH HOUR FOLLOWING THE INJECTION
Normal	Absent	None	6	2.273	2.28
Diabetes insipidus	Absent	None	11	1.955	1.9
Diabetes insipidus	Present	None	13	5.169	6.02
Diabetes insipidus	Present	$Ba(OH)_2$ solution, strong	2	6.514	6.2
Diabetes insipidus	Present	$Ba(OH)_2$ solution, medium strength	4	4.298	4.1
Diabetes insipidus	Present	$Ba(OH)_2$ solution, strong	11	2.077	2.0
Diabetes insipidus	Present	$Ba(OH)_2$ solution, medium strength	9	2.555	0.98
Diabetes insipidus	Present	$Ba(OH)_2$ solution, weak	7	1.846	1.9

per kilo of both the tuber cinereum and the mammillary bodies of hypophysectomized dogs markedly inhibit the diuresis, but on the contrary that extracts of the tuber cinereum produce an active diuresis when injected intravenously into dogs anesthetized with barbital. It is believed however that Trendelenburg's results in no way contradict the results or the conclusions drawn from them reported in this and the previous paper. The extracts, which Trendelenburg used, were chiefly those of the tuber cinereum, which was excluded as completely as possible from the extracts

used in the experiments reported in these papers. Trendelenburg found the injection of extracts of small amounts of the mammillary bodies of normal dogs to produce a slight questionable inhibition of diuresis in unanesthetized animals, results essentially in agreement with those reported in the previous paper of this series. On the other hand he did not

TABLE 4

The diuresis produced by extracts from the brains of cattle showing the precipitation of the diuretic substance by barium hydroxide. Each injection represents the extract from 480 brains. The diuresis is expressed in terms of:

$$\frac{\textit{cc. of urine per hour after injection}}{\textit{cc. of urine per hour before injection}}$$

EXPERIMENT	DIURESIS PRODUCED BY THE HALF OF THE EXTRACT, WHICH WAS NOT PRECIPITATED WITH BARIUM HYDROXIDE	pH AT WHICH THE $Ba(OH)_2$ TREATED PORTION OF THE EXTRACT WAS PRECIPITATED	DIURESIS PER HOUR DURING THE FIRST FIVE HOURS FOLLOWING THE INJECTION PRODUCED BY			PER CENT OF THE DIURETIC SUBSTANCE PRESENT IN THE PRECIPITATE	REMARKS
			Precipitate	Filtrate	Precipitate plus filtrate		
1	14.97	10.0+*	0	0	0	0	* Barium hydroxide was added until no more precipitate formed
2	8.23	10.0+*	0	0	0	0	
4		10.0 9.3 8.7	0 3.47	12.66	16.13	0 2.15	
3		8.5	0	9.77	9.77	0	
5		9.6	0	6.24	6.24	0	
10[2]		9.6 9.2	0 3.15	4.68	7.83	0 40.23	
10[1]		9.0	8.5	2.22	10.72	79.31	
15		9.1	7.035	4.85	11.885	59.19	
16		9.0	9.07	5.0	14.07	64.46	

test the diuretic properties of the extracts of the mammillary bodies of dogs with experimental diabetes insipidus or of the large amounts of the mammillary bodies of normal animals used in these experiments, both of which have uniformly produced active diuresis in unanesthetized animals.

Chemical properties. Phosphotungstic acid did not precipitate the diuretic substance from urine just as it did not precipitate it from the

extracts of the mammillary bodies or from the blood of dogs with diabetes insipidus reported previously. *Ashing* on the other hand destroyed the diuretic substance in all types of preparations, for the diuretic properties of the extracts of the mammillary bodies were completely destroyed by this procedure, 6 experiments, and those of the active preparations of blood, 4 experiments (table 2) and of the active preparations of diabetic urine (table 5) were brought within the limits of the diuresis produced by injections of preparations of the blood and urine of normal dogs, which had not been ashed. On the other hand the diuretic properties of preparations of the blood and urine of normal dogs were not materially altered by ashing (table 2, experiment 4) (table 5).

TABLE 5

The diuresis produced by preparations of urine which had been ashed compared with the diuresis produced by similar preparations which had not been ashed. Diuresis in terms of:

$$\frac{\textit{cc. urine per hour after injection}}{\textit{cc. urine per hour before injection}}$$

DONNER, CONDITION	INJECTION	NUMBER OF EXPERIMENTS	AVERAGE DIURESIS PER HOUR DURING THE FIRST FOUR HOURS AFTER INJECTION
Diabetes insipidus	Not ashed	14	5.882
Normal	Not ashed	11	2.538
Diabetes insipidus	Ashed	6	2.852
Normal	Ashed	4	2.850

Lipoid solvents, that is ether, acetone, and 95 per cent ethyl alcohol, failed to dissolve the active substance from any of the preparations, which indicates that it is probably not a lipoid.

The results of treating active preparations of blood and mammillary body extracts with *alkaline solutions* indicate that the diuretic substance is precipitated from solution by alkali at its isoelectric point and that it is labile in strongly alkaline solutions. In the case of the preparations of blood the evidence for this is not clear cut as in the case of the mammillary body extracts for the preparations were too highly colored to arrive at more than a crude estimate of the degree of alkalinity. However, the diuretic substance characteristic of diabetic blood disappeared from active preparations after treatment with so-called strongly alkaline solutions in eleven experiments out of thirteen, after precipitation with a so-called moderately strong alkaline solution in nine experiments out of thirteen, and in all cases, seven experiments, after precipitation with the so-called weakly alkaline solutions (tables 2 and 3), while the activity of six of the active

preparations was not diminished by treatment with alkali. On the other hand the diuretic substance was recovered from the precipitate of all the preparations investigated, in which it had been removed by treatment with an alkaline solution of medium strength, 4 experiments, and from two out of five of the precipitants of preparations, which had lost the active principle through treatment with strongly alkaline solutions demonstrating that at a certain alkalinity it is precipitated but that at very high alkalinity it is labile. Further evidence for the latter statement is the fact that the active preparations allowed to stand for some hours saturated with barium hydroxide produced a diuresis within the limits of that produced by the blood of normal dogs and the precipitates were inactive.

The results obtained by treating active brain extracts with barium hydroxide at definite pHs are the same in character but more specific in detail a large per cent of the diuretic substance being precipitated at a pH of 9.0, smaller per cents at pHs of 8.7, 9.1 and 9.3, and none at pHs of 8.5, 9.6, and 10.0 (table 4). The isoelectric point for the diuretic substance must be therefore in the neighborhood of a pH of 9.0. On the other hand the addition of barium hydroxide to the solution until no more precipitate formed and the solution was highly alkaline destroyed the diuretic substance (table 4, experiments 1 and 2).

SUMMARY AND CONCLUSIONS

A diuretic substance has been demonstrated in the urine of dogs with experimental diabetes insipidus, which is not present in like quantities of the urine of normal dogs.

A diuretic substance has also been demonstrated in extracts of large quantities of the mammillary bodies and thalamus of the brains of normal animals, which is not present in as great amounts if at all in like quantities of other portions of the brain, notably the parietal lobes of the cerebral hemispheres.

The diuretic substance in the extracts of the mammillary bodies is destroyed by strong alkaline solutions at room temperature, and is precipitated from solution by barium hydroxide at a pH of approximately 9.0. The diuretic substance in active preparations of the blood of dogs with experimental diabetes insipidus is likewise destroyed by strongly alkaline solutions at room temperature and is precipitated from solution by barium hydroxide in less strongly alkaline solution.

The diuretic substance in all of these preparations is destroyed by ashing, is not dissolved by ether, acetone or 95 per cent ethyl alcohol, and is not precipitated by phosphotungstic acid.

Since the diuretic substances peculiar to the mammillary bodies of normal animals and to the mammillary bodies, urine, and blood of dogs

with experimental diabetes insipidus have the same properties insofar as they have been investigated, they are presumably the same substance.

I wish to thank Dr. A. J. Carlson for generously permitting me to use his laboratories for part of these experiments and to thank Armour & Company for supplying the material for the brain extracts.

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EFFECT OF THE HEART BEAT ON THE TONUS OF SKELETAL MUSCLE

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Beginning with the time of Weir Mitchell (Mitchell and Lewis, 1886) the various investigators who have published on the knee-jerk have usually either discussed the variations occurring in the height of the knee-jerk or have published records showing these variations. Whenever the knee-jerk is recorded as vertical lines on a slowly revolving kymograph there are scarcely to be found two consecutive reactions of the same magnitude. These variations also occur when the strength of the blow delivered to the patellar tendon is constant and when the recording apparatus is very accurately adjusted. The writer has examined records of the knee-jerk from about four hundred individuals both in class work and under research conditions and all of these showed variations in height. In some of these subjects (Emery, 1925) the sensory impulses coming from the eyes and ears were excluded yet the variations in the height of the knee-jerk did not disappear. In addition to the well-known external stimuli that reinforce the knee-jerk, it has been shown that certain internal factors arising from the alimentary tract (Johnson and Carlson, 1928) and from the cerebrum (Tuttle, 1924b) also modify it. It seemed probable the heart might have an influence on the knee-jerk since the systolic discharge increases the pressure throughout the arterial system, and increases slightly the turgor of the muscles.

In order to study the effect of the heart beat on muscle tonus, as represented by the knee-jerk, simultaneous records of the carotid pulse and knee-jerk were recorded on a rapidly revolving kymograph. The carotid pulse was obtained in the usual way by means of a tambour recorder. The apparatus used for the knee-jerk was a modification of the Tuttle (1924a) knee-jerk machine, simple in construction and fairly sensitive. An electric signal magnet controlled through a mercury contact recorded the fall of the hammer. Unfortunately the release of the hammer was not automatic. It was released by an assistant at four second intervals. The records were obtained from twelve experienced subjects (men) taken from the sophomore medical class of the University. The experiments were performed under moderately quiet conditions and about three hundred reactions were taken

from each subject during a period of twenty-five minutes. The subject was adjusted before the experiment so that the hammer hit about one-half inch below the patella with sufficient force to give slightly greater than a minimal response. It should be mentioned that in order to obtain satisfactory data the carotid must be distinct and at least three alignment marks should be made for each revolution of the kymograph. From these alignment marks the knee-jerk reactions can be more accurately placed upon the carotid tracing.

When the starting of the knee-jerk reaction was carefully transferred upon the carotid tracing, by means of a compass from corresponding alignment marks, it was obvious that the larger percentage of the higher knee-jerks was occurring during the systole of the heart. The cardiac cycle was divided into two equal parts. The first half (A, table 1) was taken from the start of the anacrotic limb on the carotid tracing and included half the distance to the next anacrotic limb. The second half (B, table 1) included from the ending of period A to the commencing of the next anacrotic limb of the carotid pause. These two periods arbitrarily chosen furnish a working basis for comparing the knee-jerk during the systole and pause of the ventricles. Any reactions occurring on the division line were considered to belong to the period following or in these records to the period on the right.

It was evident from the beginning that a large number of knee-jerks would be required in order to establish an average for any given period of the heart cycle. In order to minimize the figuring and to simplify the standard of comparison the heights of one hundred knee-jerks occurring during the systole of the heart were measured. These were compared to one hundred similar measurements taken from the period of ventricular pause. This gave two hundred separate figures for each subject or two thousand reactions for the ten subjects, shown in table 1. The average height of the knee-jerk was in all the subjects greater during the systole than during the pause of the heart. The percentage increase of period A over period B (table 1) was variable, being about twenty per cent in subject six and sixty per cent in subject five with a mean average for the ten subjects of about thirty-eight per cent. Although not shown in the table subject eleven showed nineteen and subject twelve thirty-nine per cent increase in the height of the knee-jerk during period A. The variability of the height of the knee-jerk and the probable error for each subject are shown also in table 1. It will be seen that the greatest number of small reactions occurred during period B, and the greater number of large reactions occurred during period A. The variability of the height of the knee-jerk is well shown by the high probable error which is based on one hundred reactions. In measuring the records it was noticed that the subjects who had more consistent variations in the height of the knee-jerk gave a higher percentage increase during period A. This at first was thought to be due

to an error in placing the reactions and that a high knee-jerk falling on the border line between periods A and B would be more likely to be put in

TABLE 1

Variation in the height of the knee-jerk for each subject. One hundred reactions during: A, ventricular systole; B, ventricular pause

SUBJECT NUMBER		HEIGHT OF KNEE-JERK IN MILLIMETERS														MEAN (AVERAGE)	PROBABLE ERROR OF MEAN	PER CENT INCREASE DURING SYSTOLE
		0	1	2	3	4	5	6	7	8	9	10	11–15	16–20	21–25			
1	A	19	13	9	5	4	0	3	1	8	3	2	14	15	4	7.04	±0.47	58.9
	B	27	12	12	5	4	4	7	2	9	2	2	10	4		4.43	±0.33	
2	A	3	4	15	7	13	12	18	14	9	4	1				4.74	±0.16	33.1
	B	5	11	19	8	21	13	16	2	2	2	1				3.56	±0.15	
3	A	4	13	24	18	11	12	8	4	3	0	1	2			3.35	±0.16	34.5
	B	8	14	37	16	9	9	1	2	3	1					2.49	±0.13	
4	A	2	1	8	10	8	10	16	11	3	7	11	12	1		6.50	±0.23	27.7
	B	3	4	9	13	13	18	14	8	7	3	1	7			5.09	±0.20	
5	A	4	12	18	16	14	12	8	6	1	0	3	6			4.00	±0.20	60.0
	B	13	19	25	13	10	8	7	5							2.50	±0.13	
6	A	2	8	17	6	17	7	4	15	10	4	5	4	1		5.04	±0.22	20.3
	B	6	10	16	14	13	12	5	6	6	4	2	6			4.19	±0.21	
7	A	4	19	19	21	13	16	4	4							2.86	±0.12	49.8
	B	4	34	31	13	13	4	1								1.91	±0.09	
8	A	1	0	1	3	6	5	4	3	11	10	3	36	15	2	10.54	±0.31	29.6
	B	0	4	6	10	7	6	7	6	9	5	7	26	7		8.13	±0.30	
9	A	4	24	17	19	15	8	5	3	2	3					2.90	±0.14	36.2
	B	10	26	25	18	8	8	3	2							2.13	±0.11	
10	A	2	1	4	10	22	17	11	8	5	5	8	7			5.71	±0.20	26.8
	B	5	9	8	17	13	10	16	10	7	2	1	2			4.50	±0.18	
Total of period A		45	95	132	115	123	99	81	69	52	36	34	81	32	6	5.27*		37.69*
Total of period B		81	143	188	127	111	92	77	43	43	19	14	51	11		3.89*		

* Average.

period A and a small reaction placed in period B. These data were again carefully checked and similar results obtained. Thus it was evident that

the high knee-jerks were not being wrongly placed in period A. The variations in height between individual knee-jerks may depend somewhat on the strength of the stimulus as well as on the individual subject. Some subjects seem to show more variation when the blow delivered to the patellar tendon was considerable above minimal. Although the strength of the stimuli used was not studied in detail it is probable that some of the subjects would have given more significant increase in the knee-jerk during the systolic period had a strength of the blow been selected which would give a maximal percentage variation in the height of the knee-jerk.

Discussion. As stated above, the period A represents the systole of the heart beginning with the appearance of the anacrotic limb of the carotid pulse and including the first half of the cardiac cycle. The time elapsing from the striking of the patellar tendon until the muscle begins to contract and move the foot is according to Tuttle and others (1927) about 0.08 second. This time would be sufficient to cover the time required for the pulse to travel from the heart to the carotid or about 0.04 second plus the time of isometric contraction of the heart or about 0.03 second. From these figures it is seen that if the hammer hit the patellar tendon at or just prior to the beginning of ventricular contraction, the knee-jerk would fall at the beginning of period A. One can not say with certainty the exact condition of the heart without an electrocardiogram but it would seem probable that the period A includes from the beginning of ventricular contraction up to the opening of the atrio-ventricular valves and period B represents the ventricular pause. If the velocity of the pulse wave is taken to be seven meters per second the pulse wave would travel from the heart to the quadriceps muscle, a distance of about seventy centimeters, in one-tenth of a second. Thus the pulse wave would reach the quadriceps muscle in the early part of period A.

The reinforcement in muscle tonus occurring during the contraction of the heart may be due to either an overflow of nervous impulses or a mechanical effect brought about by the increased blood pressure in the muscles themselves. With the apparatus available it was not possible to take a knee-jerk record with the subject lying down and with the foot moving in a horizontal plane. This method was used extensively by Lombard (1887) and Bowditch and Warren (1890) and their records show the usual variations in the height of the knee-jerk. The data available show that whatever the causative factor or factors may be, there is present more tonus in the skeletal muscles during the systole than during the diastole of the heart.

SUMMARY

1. A large number of knee-jerk reactions have been measured during the systole of the heart and compared to similar measurements taken during the diastole of the heart.

2. The tonus of the skeletal muscles is greater during the systole than during the diastole of the heart as represented by the height of the knee-jerks.

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THE REGULATION OF RESPIRATION

XXVII. The Effect upon Salivary Secretion of Varying the Carbon Dioxide and Oxygen Content of the Inspired Air

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The experiments presented in this report were designed to determine whether gland cells, or glandular activity, are sensitive to an increase in the carbon dioxide or a decrease in the oxygen of the inspired air. This is a part of the general problem of the effect of such changes in the respired gases on other tissues and functions than the respiratory center and respiratory activity. The glandular activity under investigation was the secretion of the submaxillary gland under the influence of pilocarpine stimulation.

All of the experiments were carried out on large dogs anesthetized by the subcutaneous injection of morphine and urethane. In the earlier experiments the doses of these drugs varied but experience proved that the most satisfactory anesthesia with the circulation, that is, the blood pressure level, well maintained for eight to ten hours was obtained by the following procedure. Morphine sulphate was injected subcutaneously in the proportion of 0.35 cc. of a 2 per cent solution per kilogram of body weight. In twenty minutes to a half-hour, when the animal had vomited and began to show depression, urethane, 1 gram per kilogram in little more than enough water to make a clear solution, was injected subcutaneously. The animal was always ready for operation within an hour and a half of the time of injection of the morphine.

Cannulae were inserted in the trachea, the femoral vein, the right submaxillary duct and the left carotid artery, in this order. The cannula in the carotid was connected to a mercury manometer to record blood pressure, using 4 per cent sodium citrate solution as anti-coagulant. The lingual nerve was always cut central to the giving off of the chorda supplying the gland whose duct was cannulized. In some experiments the sympathetic supply to the gland was cut also by dividing the vago-sympathetic trunk in the neck. The cutting of the sympathetic, however, did not affect the results of the experiments with either high carbon dioxide or low oxygen (see fig. 2). A simple drop recorder and tambour were used to

record the drops of saliva falling from the cannula in the submaxillary duct. The cannula in the femoral vein was connected to a 500 cc. burette suspended one and a half meters above the vein level and containing pilocarpine, 1:100,000 in 0.9 per cent sodium chloride solution. The upper end of the burette was tightly closed with a rubber stopper through which a fine capillary tube passed to the bottom of the burette making of it a Mariotte bottle. At the beginning of an experiment the rate of injection of the pilocarpine solution was usually 2 cc. per minute. The rate of injection was decreased as soon as salivary secretion started and was maintained thereafter usually at the rate of 1 cc. per minute. This rate of pilocarpine injection in most cases gave very steady salivary secretion over long periods of time. The more rapid pilocarpine injection frequently caused a slow fall in blood pressure. Generally when the injection rate was decreased the blood pressure stopped falling, remained stationary, or rose gradually to the original level. The depressor effect of the pilocarpine appeared to be greater when an animal was markedly depressed by the anesthetic. Seldom was there any evidence of a change in heart rate as the result of the pilocarpine injection.

Three re-breathing tanks, each of more than 100 liters capacity, were used to provide the gas mixtures administered. The tanks were connected by wide tubing with a common set of respiratory valves and these in turn were connected by as short a tube as possible with the tracheal cannula making the dead space little if any greater than in the intact animal. There was an air-tight cut-off valve in the inspiratory tube and another in the expiratory tube close to the top of each tank. Proper manipulation of these valves permitted the animal to re-breathe the gas mixture in any one of the tanks. On the top of each tank was a bellows-shaped spirometer connected to a writing lever recording the respiratory movements and the rate of oxygen consumption. An excursion on the respiratory record of one centimeter represented a tank volume change of 125 cc.

One tank was kept filled with room air. The oxygen used up by the animal from this tank was replaced periodically from an oxygen cylinder. Starting with room air the oxygen content of the second tank was lowered to any desired extent by the addition of nitrogen. The expiratory tube to each of these tanks was provided with a soda-lime container to remove the expired carbon dioxide. The third re-breathing tank was like the others except for the omission of the soda-lime container. In this tank gas mixtures of high carbon dioxide content were provided by the addition of carbon dioxide to room air, usually without oxygen correction. In each case the gas mixture desired was made up accurately by water displacement, maintaining atmospheric pressure by means of a water gauge and water seal.

In general the plan of an experiment was as follows: When the operative procedures were completed and arrangements made for recording sali-

vary secretion, blood pressure, respiration, and time in seconds, the tracheal cannula was connected with the room air tank and the intravenous injection of pilocarpine was started. When salivary secretion began, usually within twenty minutes, the tracing was started. The rate of secretion was followed with a stop-watch and the rate of pilocarpine injection adjusted as necessary until the drops of saliva were falling at regular intervals. Then the valves on the room air tank were closed and those on top of another tank very quickly opened, allowing the animal to breathe a new gas mixture, low in oxygen or high in carbon dioxide as compared with room air, for a period of time varying in different experiments from one to twenty-five minutes. Then the valves on this tank were closed and those on the room air tank opened as quickly as possible. Time was allowed for the animal to recover, at least for the salivary secretion to come back to a steady rate, before again administering the same or another gas mixture. In this way were administered at different times mixtures containing from 18 to 5 per cent of oxygen and from 1 to 10 per cent of carbon dioxide. Seventeen dogs were used in all, upon which one hundred twenty observations were made. Typical tracings showing the effects of the administration of the gas mixtures are reproduced in figures 1 to 4, in each of which the percentage change in secretion has been plotted upon a base line marked off in minutes. The tracings have been reduced to one-sixth of their actual size. The results of the experiments with each gas mixture have been averaged up to the end of five minutes of administration of the gas in any case, excluding the experiments in which the administration was of briefer duration. These averages have been plotted on a percentage basis in figure 5.

The invariable effect of breathing carbon dioxide in excess of that contained in room air was an increase in the rate of salivary secretion. This increase was slight but distinct when there was 1 per cent of carbon dioxide in the inspired air; it amounted to more than 100 per cent in some instances when there was 10 per cent of carbon dioxide in the inspired air. The effect always began promptly, there being little difference in its onset when different percentages of carbon dioxide were used. Taking into account circulation time the effect must have been almost immediate when blood of increased carbon dioxide content reached the gland cells. On the other hand, the disappearance of the effect varied distinctly after the use of different percentages of carbon dioxide probably according to the time required for the blood carbon dioxide to return to normal. However, there was a distinct tendency for the secretion rate to fall below the original after the inhalation of carbon dioxide was stopped with very slow or no return to the original rate. This subsequent decrease in secretion rate was more marked and more persistent the more prolonged the administration of carbon dioxide of any percentage or the higher the

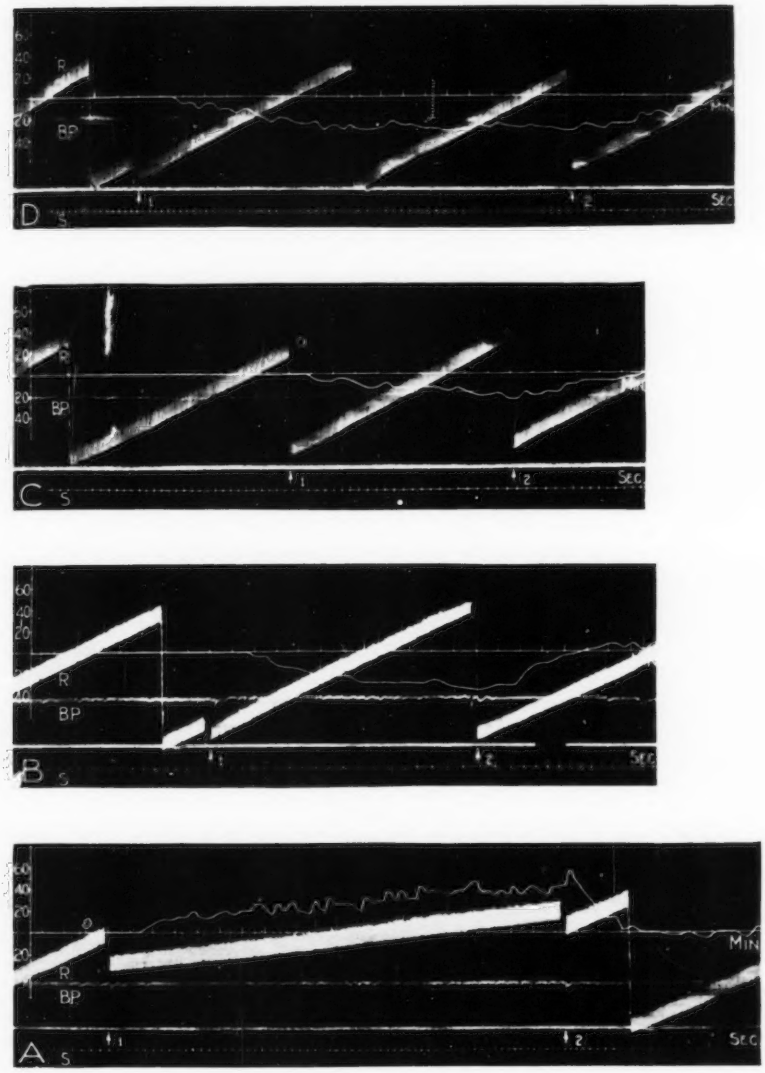

Fig. 1. *R*, respiration; *B.P.*, blood pressure, zero abscissa is base line of time record; *S*, salivary secretion. Plotted line is percentage change in secretion with percentage scale at left and base line marked off in minutes.

A, Dog 7. At *1* inhalation of 1 per cent carbon dioxide started. At *2* room air resumed.

B, Dog 7. At *1* inhalation of 15 per cent oxygen started. At *2* room air resumed.

C, Dog 6. At *1* inhalation of 10 per cent oxygen started. At *2* room air resumed.

D, Dog 6. At *1* inhalation of 10 per cent oxygen started. At *2* room air resumed.

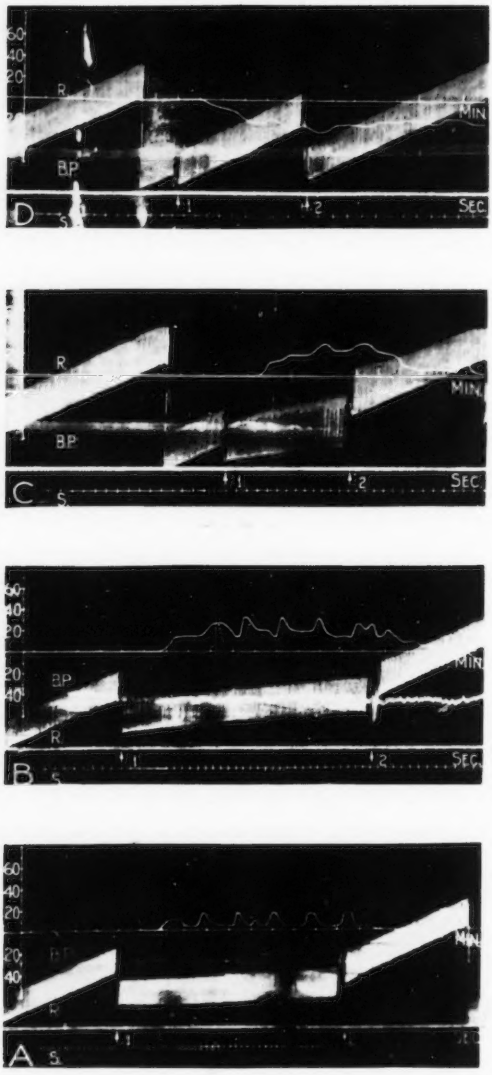

Fig. 2. *R*, respiration; *B.P.*, blood pressure, zero abscissa is base line of time record; *S*, salivary secretion. Plotted line is percentage change in secretion with percentage scale at left and base line marked off in minutes.

A, Dog 8. At *1* inhalation of 3 per cent carbon dioxide started. At *2* room air resumed.

B, Dog 8. At *1* inhalation of 5 per cent carbon dioxide started. At *2* room air resumed. Vago-sympathetic intact.

C, Dog 8. At *1* inhalation of 5 per cent carbon dioxide started. At *2* room air resumed. Vago-sympathetic cut.

D, Dog 8. At *1* inhalation of 18 per cent oxygen started. At *2* room air resumed. Vago-sympathetic cut.

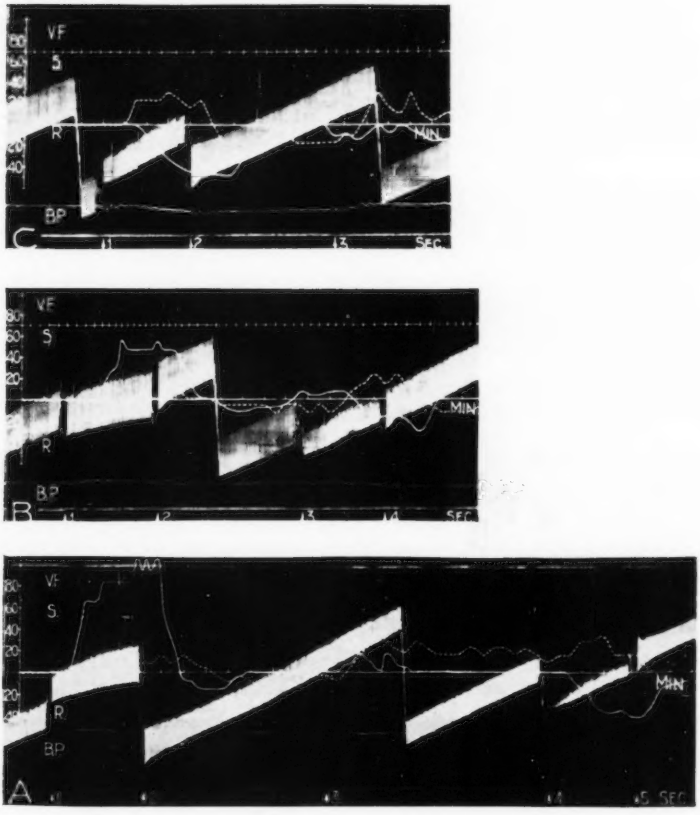

Fig. 3. *V.F.*, blood volume flow from submaxillary gland; *S*, salivary secretion; *R*, respiration; *B.P.*, blood pressure, zero abscissa is base line of time record. Solid plotted line is percentage change in secretion; dotted plotted line is percentage change in blood volume flow. Percentage scale is at left and base line is marked off in minutes.

A, Dog 14. At *1* inhalation of 10 per cent carbon dioxide started. At *2* room air resumed. At *3*, 100 cc. of 0.9 per cent sodium chloride solution injected intravenously. At *4* inhalation of 7 per cent oxygen started. At *5* room air resumed.

B, Dog 15. At *1* inhalation of 10 per cent carbon dioxide started. At *2* room air resumed. At *3* inhalation of 7 per cent oxygen started. At *4* room air resumed.

C, Dog 15. At *1* inhalation of 7 per cent oxygen started. At *2* room air resumed. At *3*, 100 cc. of 0.9 per cent sodium chloride solution injected intravenously.

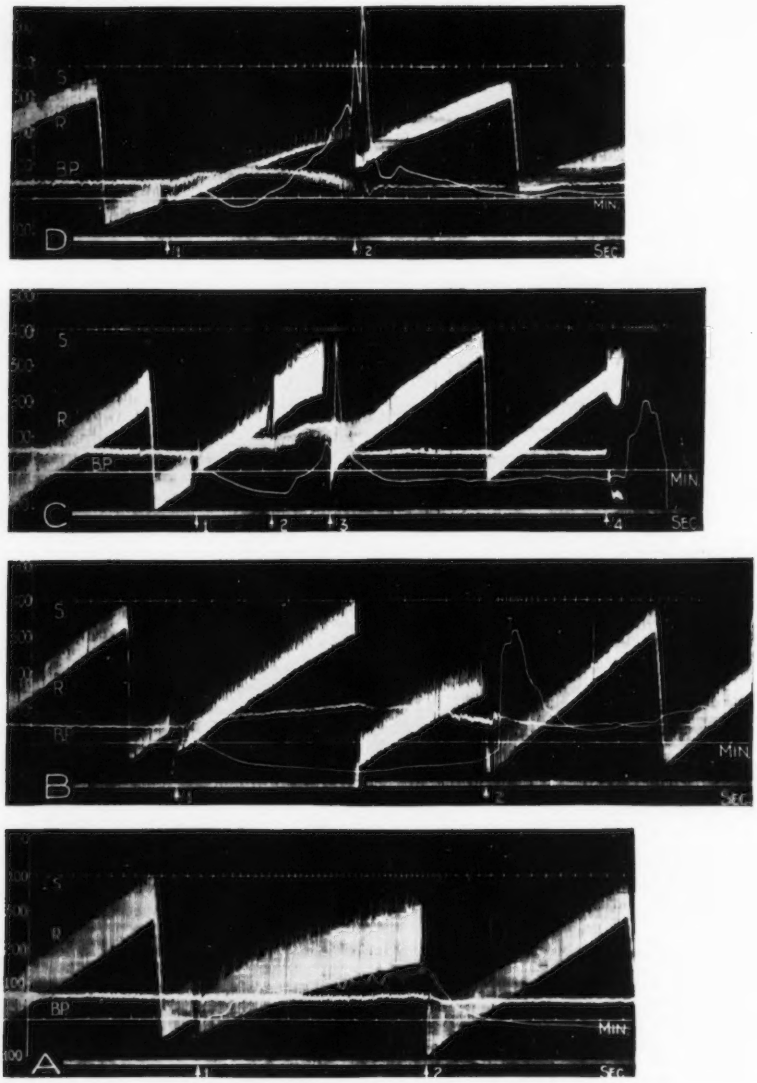

Fig. 4. *S*, salivary secretion; *R*, respiration; *B.P.*, blood pressure, zero abscissa is base line of time record. Plotted line is percentage change in secretion with percentage scale at left and base line marked off in minutes.

A, Dog 16. At *1* inhalation of 10 per cent carbon dioxide started. At *2* room air resumed.

B, Dog 16. At *1* inhalation of 7 per cent oxygen started. At *2* room air resumed.

C, Dog 16. At *1* inhalation of 7 per cent oxygen started. At *2* inhalation of 7 per cent oxygen plus 5 per cent carbon dioxide started. At *3* room air resumed. At *4*, 4 per cent sodium citrate solution injected intravenously.

D, Dog. 17. At *1* inhalation of 5 per cent oxygen started. At *2* room air resumed.

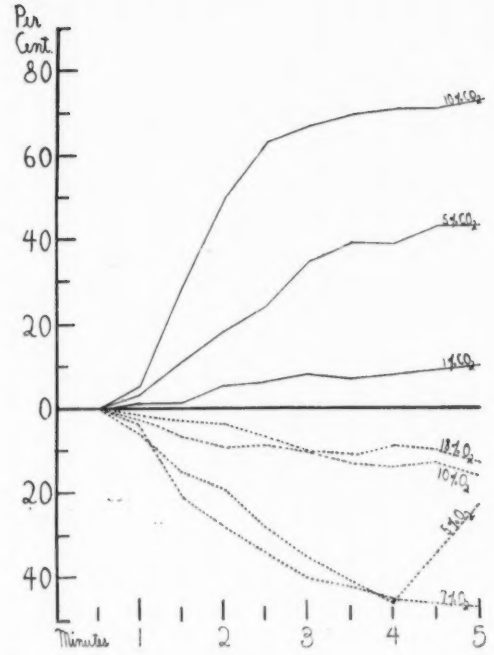

Fig. 5. Average percentage changes in salivary secretion during inhalation of air rich in carbon dioxide (solid lines) or poor in oxygen (broken lines).

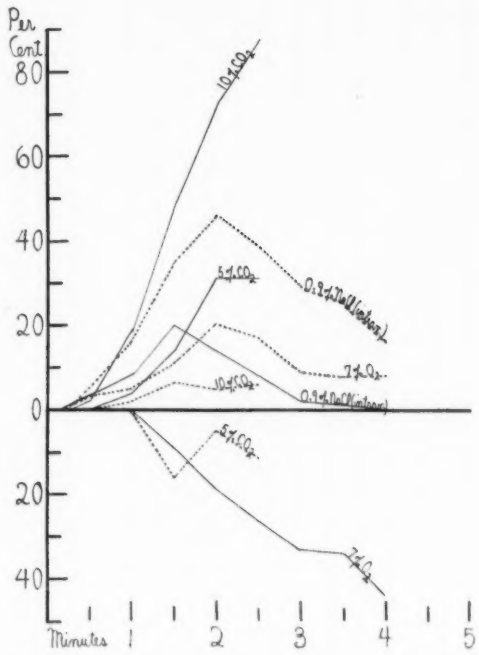

Fig. 6. Average percentage changes in salivary secretion (solid lines) and in submaxillary blood volume flow (broken lines) in those experiments in which both were recorded.

percentage of carbon dioxide breathed. Possibly despite or in addition to the augmenting effect of carbon dioxide on salivary secretion some damage was done to the gland cells. In one experiment before the pilocarpine injection was started after a twenty minute period during which there was no apparent salivary secretion 5 per cent carbon dioxide was administered for twenty minutes. No secretion resulted.

The invariable effect of breathing 18 per cent or less of oxygen was a decrease in the rate of salivary secretion, at least initially. This decrease amounted to 13 per cent on the average when 18 per cent of oxygen was breathed and sometimes amounted to 70 per cent when the oxygen in the inspired air was reduced to 7 per cent. Again the effect appeared promptly, the interval corresponding closely with that in the carbon dioxide experiments. Usually when the administration of low oxygen was stopped the secretion rate began to increase promptly recovering about half-way toward the original rate rapidly. From then on, however, recovery was often very slow.

The administration of 10 per cent of carbon dioxide was discontinued in most cases within five minutes. In three experiments it was stopped at the end of ten minutes on account of the dyspnea which developed. Sometimes with lower percentages the period of administration was more prolonged. In all of these experiments the rate of secretion continued to mount to the end of the period of administration. In one experiment (fig. 1 A) 1 per cent carbon dioxide was administered for twenty and a half minutes. At the end of five minutes the secretion had increased 18 per cent, a little more than the average effect of this amount of carbon dioxide for this time. At ten minutes the increase was 30 per cent and at the end of the administration the increase reached a maximum of 56 per cent, more than the average effect of five minutes' breathing of 5 per cent carbon dioxide. In this connection it should be pointed out that the method employed was such that carbon dioxide would accumulate in the carbon dioxide containing tank. In the experiment under consideration during the twenty and a half minutes the carbon dioxide had increased to about 3 per cent. At the same time the oxygen in the tank had decreased about 2.75 per cent. It seems as if the stimulating effect of carbon dioxide were cumulative. Certainly it more than overcame the depressant effect of the lowered oxygen.

The increasing effect of high carbon dioxide on the rate of salivary secretion in spite of simultaneous low oxygen was put to the test in another way. In experiment 110, 7 per cent oxygen was administered for three minutes. Then the animal was made to breathe a mixture containing 7 per cent oxygen and in addition 5 per cent of carbon dioxide. In a half-minute the secretion began to increase rapidly and in three minutes, a quarter of a minute after the breathing of room air was resumed, the secretion rate was 346 per cent above the original rate. The carbon dioxide seems to have been much

more effective in the presence of low oxygen than when it was administered with a normal amount of oxygen in the inspired air (see fig. 4 C).

The administration of 7 per cent oxygen was continued in four experiments for twenty to twenty-five minutes. During these administrations the blood pressure rose initially but sooner or later the rise was succeeded by a fall, gradual at first but more rapid later if the low oxygen inhalation was continued. Approximately coincident with the change in the effect on the blood pressure there occurred a change in the rate of salivary secretion. The latter began to increase and in two experiments was above the original, in one very markedly so, when the breathing of room air was resumed. In all four experiments the secretion rate continued to increase when the animal was breathing room air again and for a brief period was exceedingly rapid (see fig. 4 B). Gradually the secretion slowed down but it tended to remain above the original level. The same sequence of changes in both blood pressure and secretion, but more rapid in its progress, was observed when the gas mixture administered contained only 5 per cent of oxygen.

A very rapid secretion of saliva, similar in appearance at least to that just described after prolonged low oxygen administration, was observed when an animal was asphyxiated, whether the asphyxia was produced by clamping both inspiratory and expiratory tubes, by air embolism, or by the intravenous administration of sodium citrate (see fig. 4 C).

Apart from the coincidence already mentioned in the effect of prolonged low oxygen administration on blood pressure and secretion rate there appeared to be no relation between changes in secretion and the blood pressure level at the time of administration or the change in the blood pressure effected by the administration of any of the gas mixtures used.

Experiments were carried out on three dogs to try to show what relation if any existed between blood volume flow and secretory changes in the submaxillary gland under the influence of high carbon dioxide or low oxygen inhalation. After the submaxillary duct had been cannulized the veins in that side of the neck were dissected out and ligated in such a way that only the blood flowing from the submaxillary gland could enter the external jugular vein. This vein was cannulized. The blood flow from the gland was led thus into sodium citrate solution which it displaced drop by drop. The rate of flow of this displaced solution and the salivary secretion were recorded simultaneously while the animal was breathing room air or 5 or 10 per cent carbon dioxide or 7 per cent oxygen. From time to time 100 cc. of 0.9 per cent sodium chloride solution were injected intravenously to make good the blood lost. Sooner or later the blood clotted in the cannula but it was possible to make a number of observations on each animal before this took place. The average results of these observations are plotted on a percentage basis in figure 6 and typical tracings are reproduced in figure 3.

Blood volume flow was decreased by 5 per cent carbon dioxide and increased slightly by 10 per cent carbon dioxide. It was increased more decidedly by 7 per cent oxygen. It was increased even more markedly by the intravenous injection of saline solution. On the other hand, as we have shown, high carbon dioxide increased the rate of salivary secretion and low oxygen decreased it. Intravenous saline injection increased the rate of secretion only moderately as compared with the marked effect of carbon dioxide despite the relatively great increase in blood volume flow which it produced.

SUMMARY AND CONCLUSIONS

1. When the submaxillary gland of the dog was made to secrete at a constant rate by continuous intravenous administration of pilocarpine (1:100,000) increasing the carbon dioxide in the inspired air increased the rate of secretion and decreasing the oxygen in the inspired air decreased the rate of secretion.

2. The increase in the rate of secretion was obtained when there was 1 per cent of carbon dioxide in the inspired air. It was greater with higher percentages of carbon dioxide (to 10 per cent). It was greater the more prolonged the period of administration.

3. Following the administration of carbon dioxide when the animal was breathing room air again the secretion rate usually fell below that before the carbon dioxide was given and recovered very gradually if at all. This after-effect was more marked with the higher percentages of carbon dioxide or with prolonged administration.

4. The decrease in rate of secretion was obtained when there was 18 per cent of oxygen in the inspired air. It was greater with lower percentages of oxygen (to 7 per cent). Following the administration of low oxygen when the animal was breathing room air again the secretion rate usually recovered promptly and not infrequently increased above that before the low oxygen was administered.

5. If the administration of low oxygen (7 per cent) was prolonged the secretion rate began to increase while the low oxygen inhalation continued. In some cases this increase brought the secretion rate very much above that before the low oxygen was started.

6. The effects of high carbon dioxide and of low oxygen were obtained with both chorda tympani and vago-sympathetic cut. They appeared to be obtained independently of the effect upon the blood pressure of the artificial gas mixture administered and of the level of the blood pressure at the time of administration, except that during low oxygen inhalation the secondary increase in secretion rate and a fall in the blood pressure began at about the same time.

7. The volume flow of blood from the submaxillary gland was increased both by increasing the carbon dioxide and by decreasing the oxygen in the inspired air.

I am deeply indebted to Prof. Robert Gesell who suggested this problem for investigation and very cordially afforded me the privileges of his laboratory. His interest and advice have been of the greatest assistance throughout.

THE REGULATION OF RESPIRATION

XXVIII. Lymph Acidity and Lymph Flow During Administration of Sodium Bicarbonate and Carbon Dioxide[1]

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Much of our information on the acid base equilibrium of the body has been derived from chemical studies on the blood. The difficulties of inferring the acid-base condition of the cell from the acid-base condition of the blood are considerable for the cell is not only subjected to changes in its own acid metabolism but to the behavior of its cell membrane and capillary membranes separating it from the blood and tissue fluid. Similarly the acid-base equilibrium of the tissue fluid which forms the immediate cell environment is a resultant product of the living cell and of the blood and of the limiting membranes. The acid base properties of the tissue fluids, therefore, cannot be safely inferred from a study of the blood. Our problem is thus somewhat simplified by direct examination of various body fluids.

In an effort to learn more accurately of the acid changes in and about the neurones of the central nervous system we have followed changes in the hydrogen ion concentration of the cerebrospinal fluid during mechanical asphyxia, during administration of carbon dioxide, sodium carbonate, sodium bicarbonate, sodium cyanide and gaseous mixtures low in oxygen (Gesell and Hertzman, 1928; Hertzman and Gesell, 1928). We have now done similar experiments on the thoracic lymph of the dog.

Method. The methods employed are like those used in the studies on cerebrospinal fluid. The dogs were anesthetized with morphine and urethane and changes in acidity of the arterial blood and of the thoracic duct lymph were recorded with the manganese dioxide electrode. The thoracic duct cannula (fig. 3) was devised to register the flow of lymph in drops with a receiving and recording tambour. In the cyanide and low oxygen experiments samples of lymph were withdrawn for pH determinations with the quinhydrone electrode. This was done to establish absolute values and to avoid the disturbing effects of reducing substances on the manganese dioxide electrode during impaired oxidations. The samples

[1] Preliminary report. This Journal, Proceedings, 1928, lxxxv.

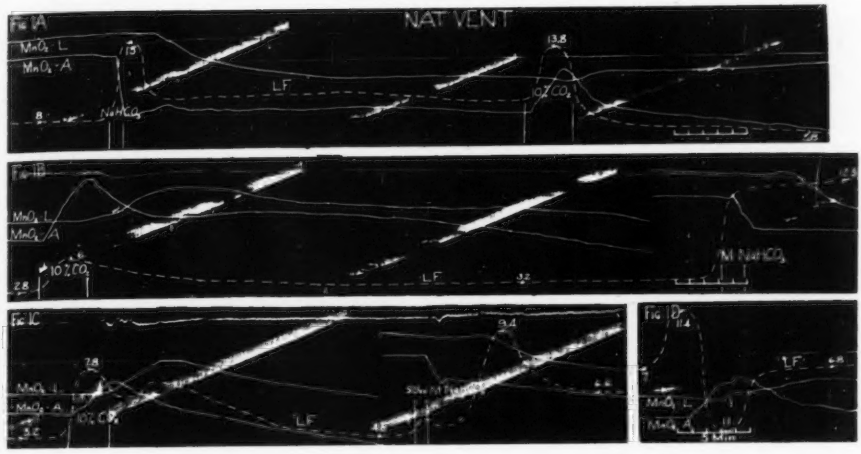
Fig 1A
NAT VENT
MnO2-L
MnO2-A
LF
NaHCO3
Fig 1B
10% CO2
M NaHCO3
LF
Fig 1C
10% CO2
LF
Fig 1D
LF
5 Min

Fig. 1

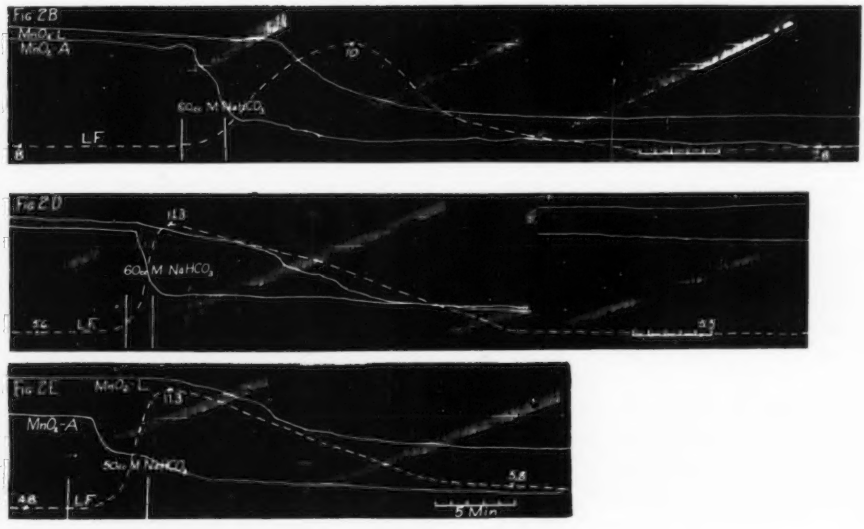
Fig 2B
MnO2-L
MnO2-A
M NaHCO3
LF
Fig 2D
M NaHCO3
LF
Fig 2E
MnO2-L
MnO2-A
M NaHCO3
LF
5 Min

Fig. 2

were taken in 1 cc. tuberculin syringes through the double rubber membrane sealing the vertical tube in the proximity of the manganese dioxide electrode and analyzed by the method previously described (Gesell, 1928). While taking the samples the free end of the electrode cannula is immersed in a beaker of saline solution. With this procedure the cannula is full of fluid at the end of sampling and will record the first drop of lymph that forms. In the experiments reported in this paper the chest was intact and ventilation was under control of the animal.

RESULTS. In figures 1 and 2 changes in the hydrogen ion concentration of the lymph and blood recorded directly on smoked paper show effects of injection of sodium bicarbonate, the administration of carbon dioxide and mechanical asphyxia. The acidity curves are marked MnO2-A and MnO2-L respectively. The original record of the flow of lymph is too

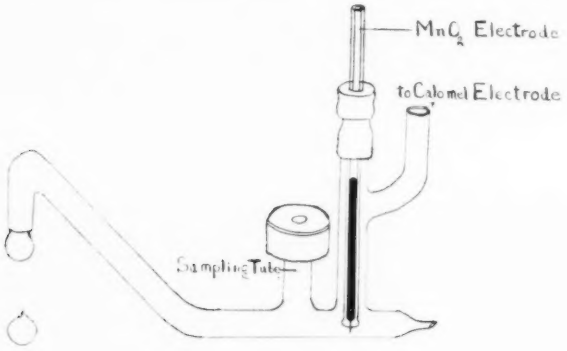

Fig. 3

indistinct for reproduction. The flow is, therefore, plotted on the smoked record—in broken line *LF*. The number of drops per minute is indicated at several points on each curve. Mean blood pressure and respiration are recorded in every observation. Five one-minute intervals are set off on the records.

On inspection of the curves attention is called to the usual "alkaline" drift of the manganese dioxide electrode. This should be taken into account in comparing hydrogen ion concentrations at the beginning and end of any procedure. It will be noted that intravenous injection of sodium bicarbonate produces a prompt and sharp increase in alkalinity of the arterial blood approximately 0.1 pH. This increased alkalinity is well maintained. There is little tendency to return to the initial acid level. After a latent period of one or two minutes varying with the animal and with the flow, the thoracic lymph turns more alkaline as well. We believe

that the alkaline change noted in the thoracic lymph at the site of the electrode occurs in the tissue spaces soon after the arrival of the alkalinized blood at the tissues; and that the latent period represents the time required for the newly alkalinized lymph to reach the electrode. Assuming an equal sensitivity of the electrodes in the lymph and blood our records indicate approximately equal changes in hydrogen ion concentration in lymph and blood. The maximum change, however, seems to develop somewhat more slowly in the lymph.

Administration of sodium bicarbonate invariably increased the flow of lymph.

Administraion of carbon dioxide turned the arterial blood sharply acid and the lymph more slowly acid. With readministration of room air the hydrogen ion concentrations diminished again.

Carbon dioxide augmented the flow of lymph as well as sodium bicarbonate. The increase was very definite and for the periods administered it was well sustained. Readministration of room air diminished the rate of flow.

Effects of mechanical asphyxia are seen in figure 1D. The blood and lymph turn acid and the flow temporarily increases. The subsequent subnormal flow will be discussed in other papers dealing with effects of impaired oxidations.

Discussion. The increase in acidity of the lymph on the administration of carbon dioxide is of course explained by the diffusion of carbon dioxide from the blood into the tissues and the lymph. That carbon dioxide leaves the blood and enters the tissues on the administration of carbon dioxide is indicated in many ways (Shaw, 1926; Brocklehurst and Henderson, 1927; Padget, 1928; Fenn, 1928; Adolph, Nance and Shiling, 1929). We have shown (Gesell, 1926) with the manganese dioxide electrode that when the acidity curves of the arterial venous blood are compared on the administration of carbon dioxide, the arterial acidity curve rises rapidly and the venous curve rises more slowly and to a lower level. The change in acidity of the blood produced by its passage through the tissues during the administration of carbon dioxide indicates a diffusion of carbon dioxide from the blood into the tissues. The slow return of the venous blood to the preadministration acidity on readministration of room air indicates the return of carbon dioxide from the tissues to the blood. We thus stated that the tissues acted as buffers to the blood by absorbing and releasing carbon dioxide. Our present experiments showing the changes in hydrogen ion concentration of the lymph supply a more direct indication of the movement of carbon dioxide and the consequent buffering action of the tissues.

Concerning the effects of intravenous injection of sodium bicarbonate on the hydrogen ion concentration of the thoracic lymph it is interesting

to contrast the decreased hydrogen ion concentration of the lymph with the increased hydrogen ion concentration occurring in the cerebrospinal fluid (Gesell and Hertzman, 1926). The acid changes in the cerebrospinal fluid were largely attributed to the semipermeability of the membranes involved in the composition of the cerebrospinal fluid, for the relative impermeability of the membranes to the basic metallic cations should prevent the immediate alkaline effect of the increased sodium bicarbonate and the free permeability to carbon dioxide should permit the acid effect of the increased carbon dioxide pressure of the blood which is known to exist (Gesell and McGinty, 1927). Accepting the view that most of the thoracic lymph comes from the liver and gastro-intestinal tract and that the blood capillaries in these regions are highly permeable it is not unexpected to find an alkaline change in the lymph accompanying intravenous injection of an alkaline salt.

If figures 1B, 1C and 2B are examined closely it will be noted, however, that the intravenous injection of sodium bicarbonate may produce a slight but definite initial increase in the hydrogen ion concentration of the lymph. This temporary effect of sodium bicarbonate is similar to the effects of intravenous injection of sodium bicarbonate on the hydrogen ion concentration of the cerebrospinal fluid. Bearing in mind that the thoracic duct is full of secretion at the time of the injection and that this already secreted lymph is subjected to the generally increased carbon dioxide pressure in the body, the increase in hydrogen ion concentration is explainable. Assuming further a differential impermeability of the thoracic duct to the basic portion of the salt the explanation of the acid change is comparable to that offered for the cerebrospinal fluid.

It is thus apparent tht if the blood capillaries are universally permeable to salts the injection of sodium bicarbonate most probably turns the immediate cell environment more alkaline. Nevertheless, both the results on the cerebro-spinal fluid and the initial acid change in the thoracic lymph indicate that the interior of the living cell may as a result of a relative impermeability of the cell membrane to metallic cations turn acid even though the immediate cell environment turns alkaline. The increased ventilation accompanying intravenous injection of sodium bicarbonate is possibly in line with such a conception.

Inasmuch as all of our observations are of relatively short duration and inasmuch as the stimulating effects of sodium bicarbonate and the depressing effects of sodium carbonate are best obtained with rapid injection a slow migration of base between blood and tissue is not necessarily precluded. The usual stimulation of respiratory movements may thus be due to the relatively greater migration of acid with respect to base. That an exchange of acid and base does occur between the blood and body with gross disturbances in equilibrium was definitely indicated (Gesell,

1919) by noting the effects of successive injections of hydrochloric acid on the alkaline reserve of the blood. Thus dividing the difference between the carbon dioxide capacities of the blood before and after injection of hydrochloric acid by the number of cubic centimeters of acid injected gave the reduction in alkaline reserve per cubic centimeter of acid.

$$\frac{51.8 - 42.4}{26} = 0.361$$

$$\frac{42.4 - 28.0}{50} = 0.288$$

$$\frac{28.0 - 20.0}{28} = 0.285$$

$$\frac{20.0 - 14.0}{30} = 0.200$$

$$\frac{14.0 - 9.7}{32} = 0.134$$

$$\frac{9.7 - 9.7}{19} = 0.000$$

These results are in agreement with those of Van Slyke and Cullen (1917) who find that the reduction of the alkaline reserve of the blood is not equivalent to the amount of acid injected. Recently Banus and Katz (1927) have found in perfusion experiments in the hind leg of the dog that some of the tissues have an effect on the acid base equilibrium of the blood in the sense that they increase the buffering capacity of the blood perfused through them after hydrochloric acid has been added to the perfusate.

It is apparent from our experiments that the lower the alkaline reserve of the blood, the less effective the administration of the acid in reducing the alkaline reserve still further. Whether or not the administration of hydrochloric acid actually reduced the alkaline reserve of the brain, thereby accounting for the augmented ventilation which occurs, we hope to determine by direct procedure.

It is of interest that under these conditions of depleted alkaline reserve administration of sodium bicarbonate not uncommonly reduces pulmonary ventilation. This is in contrast to the stimulation or absence of effects when bicarbonate is injected under more normal conditions. The reduction of pulmonary ventilation by the administration of sodium bicarbonate is thus suggestive of a rapid passage of base into the respiratory neuromechanism. The slow movement of bicarbonate taken by mouth into the tissues is generally accepted (Palmer and Van Slyke, 1917). But

whether or not base crosses the nerve cell membrane under the conditions of our experiments in a manner to account for the changes in respiration we also hope to establish by direct experiment.

The increase in flow of lymph on the injection of sodium bicarbonate is probably explained on the same basis as the increased formation of lymph which generally occurs on the injection of crystalloids. Whether the injection of sodium bicarbonate leads to increased volume flow of blood through the liver and intestines, thereby increasing the filtration pressure or whether it increases the permeability of the vessels has not been determined.

The increase in flow of lymph produced by the administration of carbon dioxide is not so easily disposed of. Landis (1928) found that exposure of the mesentery of the frog to Ringer's fluid half saturated with carbon dioxide produced no change in fluid movement and that complete saturation increased the rate of fluid movement very slightly but the wall remained impermeable to protein. Inasmuch as weak mixtures of carbon dioxide apparently accelerated the formation of lymph in the dog much more than saturated Ringer's solution did in the frog the possibility of changes in capillary permeability in the dog should be kept in mind for further study. Our main objective was not to study the changes in flow of lymph and gross changes in permeability to the large protein molecules so that changes in turbidity which may have occurred were unobserved. This phase of the subject must await further experimentation. Yet it may not be amiss to suggest another possible contributing factor to increased lymph formation, namely, increased filtration pressure in the capillaries. Bernthal (1928) has found an increased volume flow of blood through intestinal loops on the administration of carbon dioxide. Whether or not the increased respiratory movements were a source of increased lymph formation was not determined.

SUMMARY AND CONCLUSIONS

The effects of intravenous injection of sodium bicarbonate and the administration of carbon dioxide on the hydrogen ion concentration and the rate of flow of the thoracic duct lymph of the dog were studied.

The intravenous injection of sodium bicarbonate solution decreased the hydrogen ion concentration of the lymph and blood. The decrease in concentration as measured by the manganese dioxide electrode was approximately equal for lymph and blood.

Administration of carbon dioxide by respired air increased the acidity of the lymph and blood. With readministration of room air the hydrogen ion concentrations returned to their initial values.

Administration of sodium bicarbonate and carbon dioxide augmented the flow of lymph. Mechanical asphyxia had the same results.

The decreased hydrogen ion concentration of the lymph on the administration of sodium bicarbonate was explained by the free passage of salts through the highly permeable capillaries of the liver and gastro intestinal tract. These results are contrasted with the changes in acidity produced in the cerebrospinal fluid.

The increased hydrogen ion concentration of the lymph produced by the administration of carbon dioxide was explained by the free movement of carbon dioxide from the blood. These results agree with those obtained on the cerebrospinal fluid.

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THE REGULATION OF RESPIRATION[1]

XXIX. Lymph Acidity and Lymph Flow during Impaired Oxidations Produced by Cyanide

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In these experiments effects of impairment of oxidations produced by intravenous injection of 0.002 M. sodium cyanide on the hydrogen ion concentration of the lymph are studied. Changes in flow of lymph are also recorded. In one series of experiments ventilation is under the control of the animal and is, therefore, variable. In another series pneumothorax is established and ventilation is maintained at a constant volume. Changes in hydrogen ion concentration were followed with both the manganese dioxide electrode and the quinhydrone electrode. The curves established by the quinhydrone electrode are plotted on the original smoked record. The less accurate manganese dioxide electrode acidity curves have been erased. In some experiments changes in hydrogen ion concentration of the arterial blood were followed with the quinhydrone electrode. The corresponding curves are plotted on the records. In other experiments only scattered determinations were made. These are also noted on the record. The flow of lymph, pulmonary ventilation, blood pressure, time, injection, etc., are recorded as in the preceding paper. During artificial ventilation the abdominal respiratory movements are registered (see records 2D and 3A, B, C, and D,).

Results. The common result of intravenous injection of sodium cyanide during normally controlled ventilation is an initial decrease in the hydrogen ion concentration of the lymph, followed by an increase in acidity above the pre-administration level and a final return to the initial value. An example of such results is seen in figure 1A where the lymph turns about 0.05 pH more alkaline during the five minute period of injection of cyanide. During the next ten minutes it changes 0.17 pH in the acid direction and becomes 0.11 pH more acid than before injection. It then slowly recovers and within twenty five minutes reaches the original hydrogen ion concentration. In figure 1B the initial alkaline change is

[1] Preliminary report: This Journal, Proceedings, 1928, lxxxv, 373.

very small. The record indicates 0.01 pH. The subsequent acid change and recovery, however, are more comparable to figure 1A though in figure 1B it will be noted that the lymph remains distinctly more acid than normal. In figure 1C the alkaline change again is very small. The post-

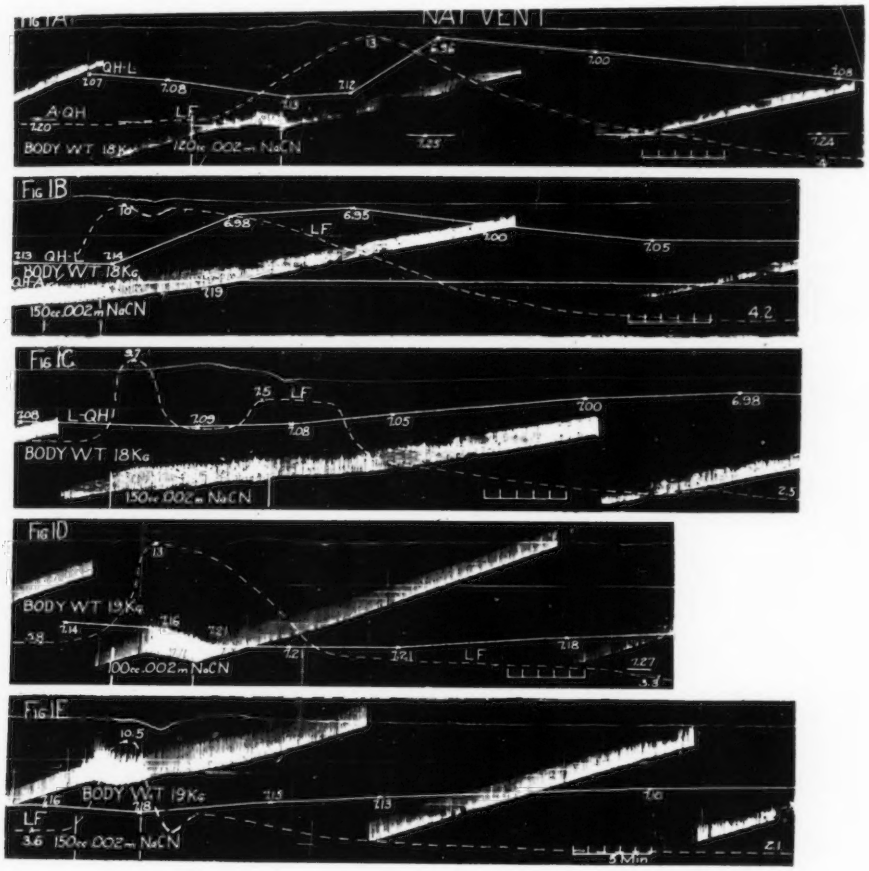

Fig. 1

administrational acid change is also small and relatively slow in developing. In figure 1D the initial change is relatively large, about 0.065 pH but the post-administrational acid swing is small and has not reached the initial level. Figure 1E is very similar to figure 1C. In figures 2A and 2B the initial alkaline swing is again more pronounced and the subsequent

acid change well marked. A less common result of cyanide injection is seen in figure 2A where the lymph turns acid though pulmonary ventilation is highly augmented.

Observations from the second series of experiments in which pulmonary ventilation is held constant are shown in figures 2D and 3A, B, C and D. Figure 2D is a typical result. It will be noted that before injection of

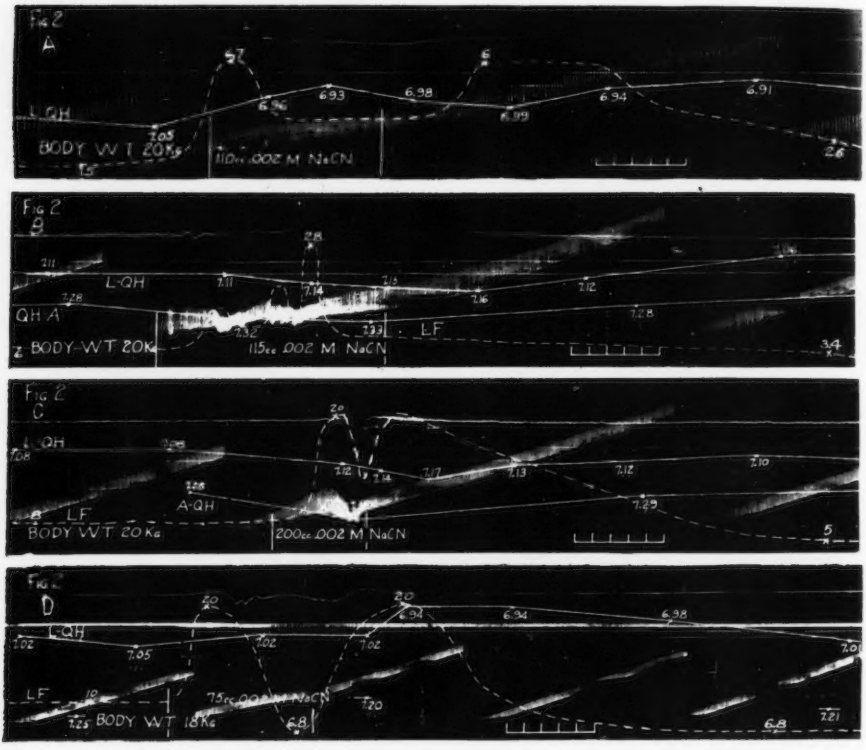

Fig. 2

sodium cyanide the lymph is turning more alkaline. This is an effect of excessive washing out of carbon dioxide produced by the artificial ventilation which was greater than the normal ventilation. (The gradient of this change is readily controlled by varying the volume of ventilation.) On administration of cyanide it will be seen that the alkaline gradient is interrupted—that the lymph turns acid (about 0.04 pH). This lasts for about five minutes, then the hydrogen ion concentration remains constant

for another five minutes and shortly after the end of injection there is a second and final increase in the hydrogen ion concentration from pH 7.02 to 6.94. This is followed by a return to the initial acidity. Figures 3A and 3B are two more examples of the double acid change in the lymph with administration of cyanide during constant ventilation. In figure 3C the first or administrational increase is missing; the second or post-administrational increase is pronounced (0.17 pH) and well maintained. Recovery is slow and at the end of two hours as shown in the continuation of record 3C the pH is still 7.12 or 0.04 more acid than the pre-administrational acidity. Record 3D is an exception. If the third determination is correct (pH 7.17) an increased alkalinity has occurred during the administration of cyanide.

Sufficient determinations on the hydrogen ion concentration were not made to establish accurate curves of changes in hydrogen ion concentration of the arterial blood. Nevertheless gross comparisons of changes in pH in the blood and lymph may be made with advantage. In figure 1A the administrational alkaline change is about equal in the blood and in the lymph. In record 1B the blood changes are insignificant and the lymph changes are large. In records 2B and C the parallelism is close. In record 2D both blood and lymph turn acid to about the same extent but return toward normal values is more complete in the lymph. In record 3A the changes in blood and lymph agree; also in record 3D.

Comparing the absolute pH values of the blood and lymph it will be seen that the blood is invariably more alkaline than the lymph. The difference varied from 0.11 to 0.29 pH. The average difference of 27 observations on 11 animals was 0.18 pH. It is assumed in these comparisons that the correction for the quinhydrone electrode is the same for lymph as for that established by Cullen and Earle (1928) for blood plasma. The absolute values given on the records are uncorrected.

Noting next the flow of lymph it will be seen that intravenous injection of cyanide invariably augments the flow. In some instances the flow increases and then decreases in a more or less progressive manner (see figs. 1A, 1B, 1D and 1E). In other records there is a secondary increase giving rise to two distinct waves of flow. The best example of this secondary fluctuation is seen in figure 2D. Other examples appear in records 1C, 2A, 2D, 3A, 3B, 3C and 3D.

Discussion. Analysis of the records indicates that the effects of administration of sodium cyanide on the hydrogen ion concentration of the lymph are different when the animal is allowed to control its ventilation and when ventilation is maintained at a constant volume by artificial means. Though variations in results occur in both series the early effects in general are decreased hydrogen ion concentration during normal ventilation and increased hydrogen ion concentration during constant ventilation. This

agrees with our findings on the cerebrospinal fluid (Gesell and Hertzman, 1928). The initial decreased hydrogen ion concentration during normally controlled ventilation is attributed to excessive elimination of carbon

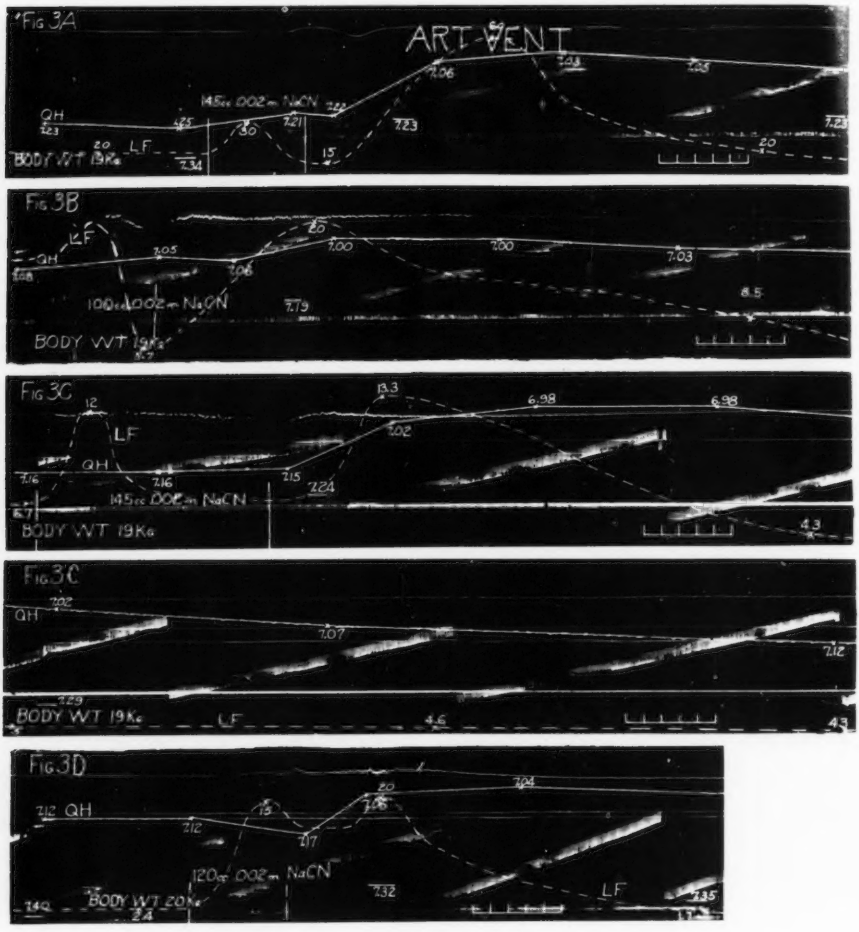

Fig. 3

dioxide during the period of augmented ventilation. The initial increased hydrogen ion concentration occurring in the absence of augmented ventilation in the pneumothorax series is attributed to the acid effects of the highly oxidized condition of the hemoglobin on the hydrogen ion concen-

tration of the blood, to the impaired elimination of carbon dioxide resulting from a broken coördination of the dual function of hemoglobin at the tissues and in the lungs, and to an increased formation of fixed acid. The final increase in acidity of the lymph with normal and artificially controlled ventilation is attributed to a sudden restoration of the oxidative mechanism of the cells occurring at a time when there is an increased store of anaerobic metabolites awaiting oxidation. The result of this combination is increased production of acid. The final recovery to normal acid values is then due to the elimination of the excess carbon dioxide and a restoration of the normal base by the disappearance of the accumulated lactic acid.

If the number of experiments that we have done is sufficient to make a safe comparison it would appear that both the initial alkaline change and the post-administrational acid change is greater in the cerebrospinal fluid than in the thoracic lymph during normal ventilation (Gesell and Hertzman, 1928). We hope to analyze this phase of the subject in a later paper.

With regard to the changes in rate of flow of lymph it was noted that cyanide not only increased the flow of lymph but increased the turbidity as well. Lymph which was almost clear before administration of cyanide turned very cloudy and slightly blood tinged soon after injection. As the flow again subsided the lymph acquired its original appearance. The same results (Gesell, 1928) were obtained on the administration of low oxygen mixtures. The effects are thus produced by either a disturbance in the oxidative mechanism with abundance of oxygen or by lack of oxygen with the oxidative mechanism intact. The changes in turbidity of the lymph are thus attributed to changes in permeability of the capillary walls resulting from direct effects of impairment of oxidations. The findings are in line with the postulate that any form of tissue abuse tends to increase the permeability of the capillaries (Gesell, 1919). The results agree with the direct observations of Landis (1928) on the capillaries of the frog, that filtration of fluid through the capillaries and the permeability of the capillary walls are increased by lack of oxygen.

That the changes in flow of lymph as well as the changes in composition of lymph are a function of the changes in permeability of the capillaries is highly probable. Yet our experiments which were not designed to study the factors of flow of lymph include other variables. To what extent changes in capillary blood pressure influenced the results we cannot say. Neither can we say that the intensity of the respiratory movements was without effect. It will be noted, for example, especially in the experiments with constant ventilation that a rough coincidence between rate of flow and activity of respiratory muscles obtains (see fig. 2D). Sodium cyanide elicites a primary stimulation of respiratory movements, yet despite the continuation of injection, stimulation gives way to depres-

sion. This depression changes back to a secondary stimulation after the close of injection during recovery as oxidations again improve. Similar results are seen in figures 3B, C and D. While the maximum flows and maximum activity of the respiratory muscles occur together it is by no means as accurate to state that a parallelism exists. In several of the records the post-administrational flow has reached normal values though respiratory movements are still above normal. We have not yet controlled this phase of the subject with the use of curari.

It is not without interest that steady injection of sodium cyanide if not too rapid (for irregular and rapid injection see figs. 3B and C respectively) may produce a well maintained increase in respiratory movements whereas injection during constant ventilation may produce the primary stimulation which is followed by depression. The secondary stimulation noted in figure 2D occurs only after the end of injection when recovery of oxidation is in progress. We may be justified in concluding that the absence of depression during administration of cyanide with the chest intact is due to the beneficial effects of hyperventilation. If true we must further conclude that the respiratory mechanisms of the body are operating on a relatively small margin of safety. If we may assume that depression of oxidations by cyanide is primarily a disturbance in the oxidative mechanism of the cells and is not related to the supply of oxygen (which is presumably abundant) then it may be logical to conclude that the beneficial effects of hyperventilation in maintaining respiratory activity are due to a blowing off of carbon dioxide. It is likely that the blowing off of carbon dioxide enhances oxidations.

SUMMARY AND CONCLUSIONS

Effects of intravenous injection of 0.002 M sodium cyanide on the hydrogen ion concentration of the thoracic lymph were studied with the quinhydrone electrode.

In one series of experiments pulmonary ventilation was under the control of the animal. In another series pneumothorax was established and kept constant by artificial means.

During normal control of ventilation cyanide commonly decreased the hydrogen ion concentration of the lymph during the period of impaired oxidations and stimulation of respiratory movements. With return of oxidations the hydrogen ion concentration increased above the normal and subsequently returned to this level.

During constant pulmonary ventilation cyanide increased the hydrogen ion concentration of the lymph in two stages—first during the period of impaired oxidations and second during the return of oxidations. The second increase was followed by return toward normal values.

Changes in the hydrogen ion concentration of the arterial blood were

followed in a portion of the experiments. In some instances the changes in acidity of the lymph and blood were parallel, in others they were not.

Assuming the same correction for the quinhydrone electrode for blood plasma and for lymph, plasma was found to be 0.11 to 0.29 pH more alkaline than lymph.

Cyanide markedly increased the flow of lymph. This increase in flow was accompanied by increasing turbidity and increasing content of blood cells. Recovery from injection to normal flow was associated with decreasing turbidity and blood cell content.

Steady injection of cyanide produced a relatively well maintained stimulation of respiratory movements with the chest intact as compared with the early depression during constant ventilation.

The initial decrease in hydrogen ion concentration of the lymph during normal control of ventilation is attributed to the blowing off of the carbon dioxide by the augmented ventilation. The initial increase in hydrogen ion concentration during constant ventilation is attributed to a highly oxidized condition of the hemoglobin, to a broken coördination of the dual function of hemoglobin and to an increased formation of fixed acid. The final increase in acidity in both series of experiments is attributed to a return of oxidations imposed on an accumulated store of anaerobic metabolites.

A causal relation between rate of flow of lymph and permeability of the capillaries dependent on the state of oxidations in the capillary walls is indicated.

The early depression of respiratory movements on the injection of cyanide during constant ventilation as contrasted with the well maintained ventilation during normal control indicates the beneficial effects of hyperventilation and the narrow margin on which the respiratory mechanism may be operating.

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REGULATION OF RESPIRATION

XXX. The Effects of Mechanical Asphyxia and Administration of Carbon Dioxide, Sodium Carbonate, Sodium Bicarbonate and Sodium Cyanide on the Reflex Response of the Anterior Tibial Muscle of the Dog

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To further elucidate the mechanism of the control of respiration it seemed desirable to compare the effects of various conditions related to the control of respiration upon respiratory movements and upon the reflex response of a skeletal muscle. The conditions selected for study were mechanical asphyxia, high alveolar oxygen, increased alveolar carbon dioxide, intravenous injection of sodium carbonate, sodium bicarbonate, and sodium cyanide.

Method. The work was performed on dogs under morphine sulphate and urethane anesthesia. Special precaution was essential to keep the anesthesia deep enough to prevent pain to the animal and yet to keep it light enough to avoid serious depression of the reflex activities of the cord. The animals averaged about twelve kilograms in weight.

The reflex studied was the contraction of the anterior tibial muscle elicited by single induction shocks applied to the central end of the ligated tibial nerve. This reflex was selected as the muscle is easily isolated and prepared for study; also the reflex arc involved is a relatively simple one, involving, probably, only a two neurone chain (Sherrington, 1906). It is thus more easily stimulated and is more constant in its response than one involving several synapses.

The animals were connected by tracheal cannula with rebreathing tanks and arterial and venous cannulae were inserted for recording blood pressure and making intravenous injections. The tendon of the anterior tibial muscle was then exposed and freed from its insertion for recording its contractions. The sciatic nerve was next exposed along its course in the thigh and the nerves to the hamstring muscles severed as they left the main nerve. The tibial nerve was ligated as far distally as possible from this exposure and loosened from the surrounding tissues for stimulation. The leg was fixed in a horizontal position by tying the quadriceps and

Achilles tendons to horizontal bars. In this way, very little of the movement of the animal as a whole was transmitted to the leg and so recorded. The muscle was slightly loaded but not after-loaded.

Uniform stimulation of constant interruption was supplied with a motor driven disk contact breaker placed in the primary circuit of an induction coil. The secondary shocks were led through a power vacuum tube thus eliminating the break shocks. Electrodes from this tube were applied to the tibial nerve central to the ligature. Submaximal stimuli were used in most cases so that either increased or decreased responses could be recorded. In some few instances subminimal stimuli were used. The strength of the stimulus was kept constant throughout a single observation, but was varied when necessary by changing the distance between the primary and secondary coils of the inductorium. The strength of the current whenever given on the graphs is expressed in centimeters of separation from complete superimposition of the secondary over the primary coil. The rate of stimulation of about twelve per minute was chosen because this rate gave a fairly uniform response over a prolonged time.

In some of the later experiments, instead of applying the electrodes directly to the nerve, a large indifferent electrode was placed over the neck of the animal and a small stimulating electrode was inserted into the skin on the plantar surface of the foot. This was found to be the more satisfactory method of the two, as it avoided exposure of the stimulated nerve to drying which tended to decrease the response with a constant current and reduced the operative procedure.

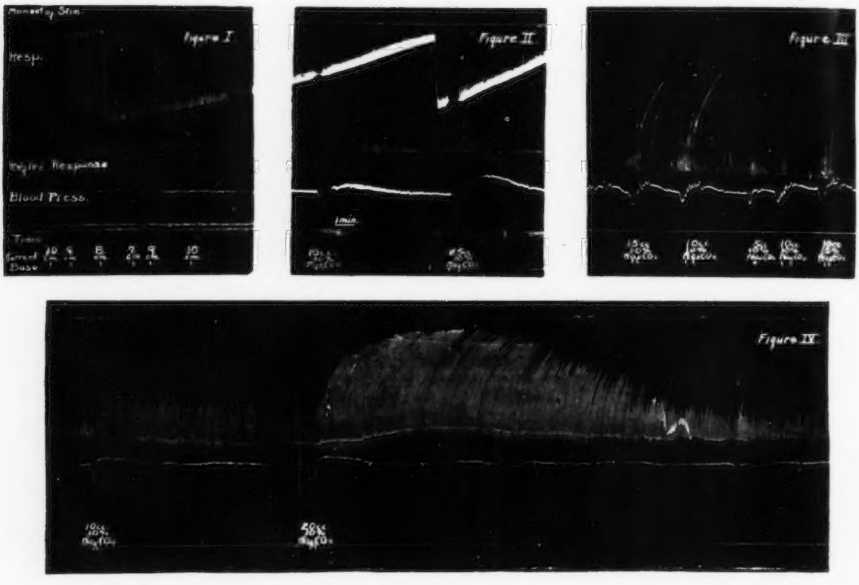
Results. Mechanical asphyxia produced by clamping of the trachea was followed in most animals by a progressive decrease in contraction of the anterior tibial muscle to reflex stimulation (fig. 11). On de-occluding the trachea the recovery was at first irregular, but later improved progressively until the response was back to normal. In some cases the recovery contractions would temporarily go to a height above normal. In a considerable number of the animals, however, there was no decrease in the muscular contractions. On the contrary, after a short interval the response increased until the animal was again allowed to breathe (figs. 9 and 10). The response then steadily declined to its original amplitude. It was observed that each particular animal gave one type of response only.

Pure oxygen at atmospheric pressure, even when administered over a considerable length of time, neither increased nor decreased the reflex response. This is in accord with the works of Mathison (1910) who says, "In no experiments have I seen any evidence in favour of the view put forward by Bethe (1906) that under normal conditions the administration of oxygen lessens the excitability of the spinal centers." The difference in results, however, may be due to a slight difference in conditions of the

animal for Mr. Gay finds (unpublished) that high oxygen depresses the reflexes.

Administrations of two to ten per cent carbon dioxide mixtures were not constant in their effect upon the reflex response of the anterior tibial muscles; but administration of a 20 per cent or stronger mixture in oxygen almost invariably inhibited the response. Figure 8 shows a decreased response obtained with only 10 per cent.

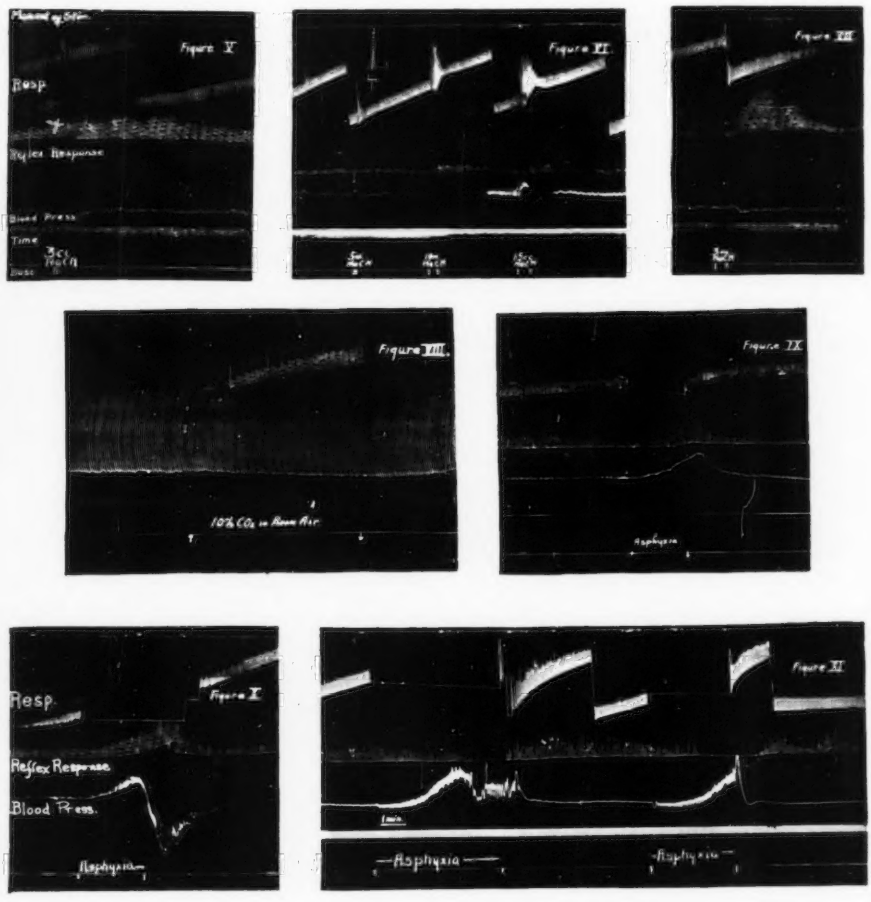
Sodium carbonate when administered intravenously in doses of five to twenty cubic centimeters of a 10 per cent solution gave an increased response which was proportional to the amount of the salt given (figs. 2, 3, 4).



The reaction to the larger doses was sustained over a period of several minutes. The return to normal was slow and gradual. Figure 3 represents a case in which a subminimal stimulus was used. The reflex response was elicited only immediately following each of the repeated injections. In the other instances (fig. 4) submaximal stimuli were used. The rate at which the solution was injected also affected the results. When given slowly or in more dilute solutions the effects were slight or absent. These effects of variation of rate of injection on the reflex response of the anterior tibial muscle are similar to those on respiratory movements (Gesell, 1929).

Sodium bicarbonate in similar or much larger injections produced no

constant effect. In some few cases there was a very slight decrease while in others there was a slight increase. Still others showed no change at all. Most of the variations fell within what might be considered normal fluctuations.



Sodium cyanide was used in a solution of M/100 concentration. Here again there was a variation in the response of the animals, but if the dose was sufficiently increased there was an increase in the response (figs. 5, 6, 7).

Discussion. The variable changes in the reflex response during mechanical asphyxia suggest that two forces are at work: one tending to

increase the response, the other tending to decrease it. That the results are not related to the moment of onset of asphyxia is evident from the records. In figure 11 the first asphyxiation was started at the end of expiration as it also was in figure 10; yet in the first case a decreased reflex response occurred, while in the second an increased response occurred. In the second half of figure 11 where asphyxia was started at the end of inspiration, a decrease also occurred. Thus in the same animal the same effects were produced regardless of the moment of occlusion of the trachea. It is, therefore, advisable to look for other causes of the variable effects of mechanical asphyxia.

Since mechanical asphyxia leads to a reduction in the oxygen supply to the tissues and an accumulation of carbonic and fixed acids in the body, it is conceivable that the effect on the reflex response is a resultant of these two factors. The depressing effects of carbon dioxide found in these experiments agrees with Verworn's observations that strychnine convulsions of the frog are stopped by an atmosphere of carbon dioxide, and could account for the depression of the reflex response during mechanical asphyxia. The augmentation of the reflex response might then be explained by the effects of lack of oxygen.

Sherrington (1910) states, "A certain degree of asphyxiation much favors the elicitation of the scratch reflex." Graham Brown (1909) too has shown that, in guinea pigs under ether narcosis, the stopping of respiration by holding cotton wool over the mouth and nostrils will bring out the scratch reflex when not present before. Kaya and Starling (1909) state that the increase in reflex response on asphyxiation is primarily due to decrease of oxygen and not an increase in carbon dioxide, as they were able to obtain similar results with 4 or 8 per cent oxygen in 92 to 96 per cent nitrogen. My experiments show the augmenting effects of impaired oxidation produced by the administration of sodium cyanide. It seems then that in asphyxia either the effects of impaired oxidations or of acid excess may predominate. In the former we obtain an increase in response while in the latter a decrease ensues.

The question now arises as to the nature of the effect produced by the deficient oxygen or the increased acid which results in these changes. Porter and Hart (1923) have shown, by using a very small muscle and stimulating it reflexly with a constantly increasing current, that the response increases by steps similar to those obtained from a similar procedure on a nerve-muscle preparation. They consequently conclude that the acting synapses respond in an all-or-none manner. With increasing stimulus, more synapses are brought into activity and consequently more nerve fibers as well as muscle fibers are stimulated. Their records are similar to my figure 1, which, however, was obtained by increasing the current by steps, not gradually. This figure is included merely to show

the regularity of reflex responses which can be elicited as well as their variations with changes in the strength of the stimulus. It is thus possible that with oxygen deficiency some factor comes into play which throws more synapses into activity. In the case of excess acid the opposite process may occur. It should be pointed out, however, that our experiments do not definitely establish the fact that the action of mechanical asphyxia, cyanide, carbon dioxide, etc., is on the synapses. It may be on other parts of the reflex arc as well.

Lennox and Cobb, 1928, in a recent study of epilepsy, have made numerous clinical observations which are also of interest in light of the above findings. They report first, that, in patients frequently and rather regularly having seizures, large oral administration of alkali to the patients resulted in a great increase in the frequency of the seizures. Later, they also state that "in certain patients having frequent, slight attacks, these can be induced by decreasing the oxygen content of the respired air. This is done by having the patient rebreathe, the expired CO_2 being absorbed." A similar degree of anoxemia with an increasing percentage of CO_2, however, would not induce an attack. On the contrary they found that if the patient breathes air not deficient in O_2 but having a high percentage of CO_2, the seizures could be stopped.

Under these three conditions, then, the seizures of epilepsy respond in the same manner as did the anterior tibial muscle to reflex stimulation. It is assumed that with oral administration of alkali, base slowly enters the tissues (Gesell, 1929) and tends to turn them alkaline in contrast to the momentary acid effect of rapid intravenous injection of sodium bicarbonate. This strongly suggests that the effects produced in the epileptic patient were due to a change in the reflex arc.

Sheldon (1928) has also reported on the use of CO_2 for the control of hiccup. He records a number of cases in which it was administered along with air or anesthetic gases in concentration of about 5 per cent for periods varying from one to twenty minutes with resultant relief from the spasms. His explanation of the effect was that with maximum stimulation of the respiratory center "the reflex or abnormal or lesser stimulation resulting in hiccup will be ineffective and that respiration without interruption may continue."

I have also seen CO_2 administered to patients with hiccup with almost immediate relief. Here, though it was given in a mixture of 80 per cent CO_2 and 20 per cent O_2, only three to five breaths were given and the patient would usually go on breathing normally for some time. In view of my observations on the dog it seems possible that the hiccup stops, not because the continued stimulus to the respiratory center is intensified and so the abnormal and lesser stimulus is without effect, but because of a decrease in the excitability of the reflex arc involved in the hiccup.

If we now compare the reflex responses with the behavior of the respiration under the same conditions, it is seen that the two do not act similarly in all cases. In fact, where one is increased the other may be decreased. A brief summary of these facts shows that: high alveolar carbon dioxide gave decreased reflex response and increased respiration; low alveolar oxygen gave increased reflex response and increased respiration; sodium cyanide gave increased reflex response and increased respiration; sodium carbonate gave increased reflex response and decreased respiration; sodium bicarbonate gave indefinite results. The effects of sodium bicarbonate on respiration are also variable, depending on the rate of injection. Impairment of oxidation as occurs from low alveolar oxygen and sodium cyanide thus appears to have the same effect upon respiratory movements and spinal reflex activity. The movement of carbon dioxide, however, between the blood and tissues, appears to exert opposite effects upon respiratory movements and spinal reflex activities.

SUMMARY

A method for the observation and study of a simple reflex response in the anesthetized dog is described. A method of uniform stimulation at infrequent intervals is also given.

It is pointed out that low alveolar oxygen tends to increase the spinal reflex response.

Sodium cyanide similarly is found to result in an increased response when administered intravenously.

High alveolar carbon dioxide is found to give a decreased reflex response when administered in moderately high concentration.

Sodium carbonate in moderate doses, especially if injected rapidly, is found to cause an increase in the reflex response.

Sodium bicarbonate even in large and rapid injections gave no characteristic change and in many cases no effect was produced.

Mechanical asphyxia is shown to cause either an increased or decreased response, the difference probably depending upon whether oxygen deficiency or acid excess (carbon dioxide and fixed acid) predominates.

Rebreathing pure oxygen at atmospheric pressure was found to exert no noticeable effect upon the spinal reflex response.

The point of action of mechanical asphyxia, carbon dioxide, sodium carbonate, sodium bicarbonate and cyanide on the reflex arc was not definitely established.

Respiratory movements and reflex activity were observed to respond similarly to those conditions producing impairment of oxidation, but oppositely when the movement of carbon dioxide between the blood and tissues is altered.

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